Evaluation of BSCB-2 probiotics effects on male rats DAI and colon histology

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Abstract

Probiotics were either live microbes or microbial products used in various food supplements, feeds and drugs for humans and animals. Probiotics improved the catabolic and anabolic activities directly correlated with the microbiomes development. Ulcerative colitis is one of the most common types of intestinal bowel disease which developed colon and rectal inflammation. Relapsing of UC evidenced in chemical drugs treated patients whereas currently probiotics were used to treat colitis. DSS used to induce colitis in male albino wistar rats (n=6). *Bacillus coagulans* (BSCB-2) probiotics were used to treat colitis in rats. DSS colitis group showed degenerated colon tissues resulted in increased DAI and gross rectal bleeding whereas DSS+*Bacillus coagulans* group relapsing the normal histology of colon resulted in significantly reduced DAI and rectal bleeding. Our study concluded that BSCB-2 probiotics cure and retain the colon histoarchitecture in DSS colitis induced male rats.

Keywords

Probiotics, DSS, DAI, colon

1. Introduction

Probiotics were either live microbes (bacteria, fungi) or microbial products (primary and secondary metabolites) used in various food supplements and drugs for humans and animals (Hill et al., 2014). According to FDA (Food and Drug Adminstration) regulations, a single strain probiotics or combination of probiotics used to treat various human ailments. Probiotics can easily as well as orally administer into the host (Sanders 2015) which directly enhances the immune system. Probiotics improved the microflora in host intestines which significantly influences the catabolic and anabolic activities directly correlated with the microbiomes (Ozen and Dinleyici, 2015; Venugopalan et al., 2010). Various organization (FAO and WHO) experts reported the standard regulations for probiotics development which showed generic safety and non pathogenic properties (FAO 2006).

Inflammatory bowel diseases (IBD) were characterized by colitis and other symptoms such as intestinal inflammation, blisters in intestinal crypts and also infiltrated WBC cells in the colon tissues (Venturi et al., 1999). Ulcerative colitis is one of the most common types of IBD which developed colon and rectal inflammation. A wide spectrum of antibiotics was used to treat human colitis but relapsing of UC occurs in 40% of treated patients (Axelssonand and Ahlstedt, 1993). Currently as an alternative for antibiotics, different bacterial probiotics strains such as *Lactobacillus* spp., *Streptococcus* spp. and *Bifidobacterium* spp. were used (Sivamaruthi 2018).

Rat gastrointestinal tract contains diversed microbiota which constitutes >500 bacterial species while probiotics oral administrate positively favored the bacterial growth and colon function (Guarner and Malagelada, 2003). Rat fecal material constitutes majorly by bacteria (Saxami *et al.*, 2012). For colitis induction, DSS is the most commonly used chemical for animal studies (Shalini Gnanam *et al.*, 2020) due to their toxic nature on gastrointestinal cells which directly acts on intestinal lumens and triggers the luminal antigens resulted in intestinal inflammation (Perse and Cerar, 2012; Solomon *et al*., 2010).

Due to the infiltrated immune cells in the colon mucosal regions, the discharged fecal consistency and colon histological changes (Wirtz *et al.* 2007; Martin *et al.* 2014b) were considered as the basic parameters in this study. Probiotics, *Bacillus coagulans* (BSCB-2) isolated from marine shrimp *Penaeus monodon* reported in our previous works (Kolanchinathan *et al.*, 2017). The objective of this study is to analyze the effect of BSCB-2

probiotics on colitis induced male albino wistar rats: DAI, rectal bleeding and colon histology.

2. Materials and methods

2.1. Experimental animals

Rattus norvegicus (150 to 200g), adult males were procured from Indian Institute of Science (Bangalore) were maintained in a controlled environment 12h dark/light cycle at 25±2°C. Feed were purchased from Sai Durga Feeds and Foods (Bangalore) and water ad libitum. For 15 days the acclimatized rats were quarantined before the commencement of the experiment. Animal maintenance and experiment were carried in based on the ethical guide lines as BDU/IAEC/2017/NE/15/Dt.21.03.2017.

2.2. Rat treatments

5% dextran sodium sulphate (DSS) dissolved in water was orally administered into twelve rats between 1st and 7th day for colitis induction. Control group (n=6) rats were maintained separately without any drugs. For colitis treatment, *Bacillus coagulans* (BSCB-2) probiotics were orally administered everyday throughout the experiment (Shalini Gnanam *et al.*, 2020).

2.3. DAI index and rectal bleeding analysis

Control and treated group rats fecal droppings and their consistency were analysed. Stool dropping with blood and nature were also observed. According to Yu *et al.* (2017), the DAI scores for typical pellet like stool consistency, liquefied stool consistency and diarrhea in rats. For rectal bleeding score (Murano *et al*., 2000), the discharge of blood as spots, streaky and gross discharge was observed. Table 1 showed scoring value for DAI and rectal bleeding in colitis induced and treated rats. Data were interpreted with SPSS tool.

Table 1. DAI and Rectal bleeding scoring in rats based on the following observations.

Observations	**Disease activity index score**	**Observations**	**Rectal bleeding score**
Normal pellets	0	No bleeding	0
Semi-formed and pasty pellets	2	Slight bleeding	1
Diarrhea	4	Bleeding inbetween or edges of stool	2
		Gross bleeding	4

2.4. Histological analysis

On the fourteenth day, the rats were sacrificed and colons were dissected for histological analysis. Colon tissues were fixed in 10% formalin and dehydrated through a series of alcohol and cleared in xylol. Cleared tissues were embedded in paraffin wax. Leica ultra microtome was used to make 5µm thickness sections and stained with Haemotoxylin and Eosin (Humason 1979). The sections were mounted in DPX, studied and photographed using photomicrography unit.

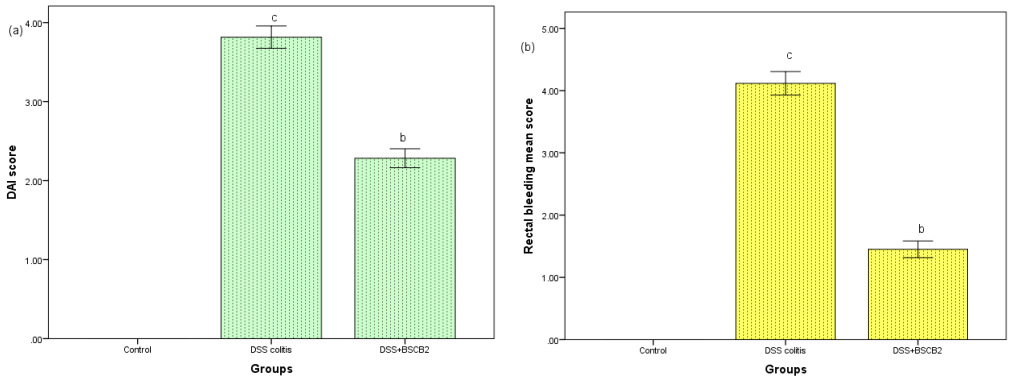
3. Results

3.1. DAI and Rectal bleeding analysis

According the scoring methods, the severity of the disease condition was scored. In control group, the fecal droppings were normal as pellets and also no evidences of rectal bleeding during the experiment. In DSS colitis induced group, rats showed severe rectal bleeding along with diarrhea throughout the experiment. In DSS+BSCB2 group, colitis treated with *Bacillus coagulans* probiotics rats showed slight rectal bleeding along with pasty pellets. The score results were presented in the bar diagram whereas control group scored as zero, so there was no representation of control group bar (Figure 1a & b).

The mean (±SD) DAI score for Group II (DSS colitis) and Group III (DSS+BSCB2) were 3.816±0.172 and 2.283±0.147 respectively. Similarly, no rectal bleeding scores for control group eventually no bar representation. Rectal bleeding score for Group II and III were found as 4.116±0.231 and 1.450±0.164 respectively.

Figure 1(a & b). DAI and rectal bleeding scoring in control, colitis induced and probiotics treated groups.



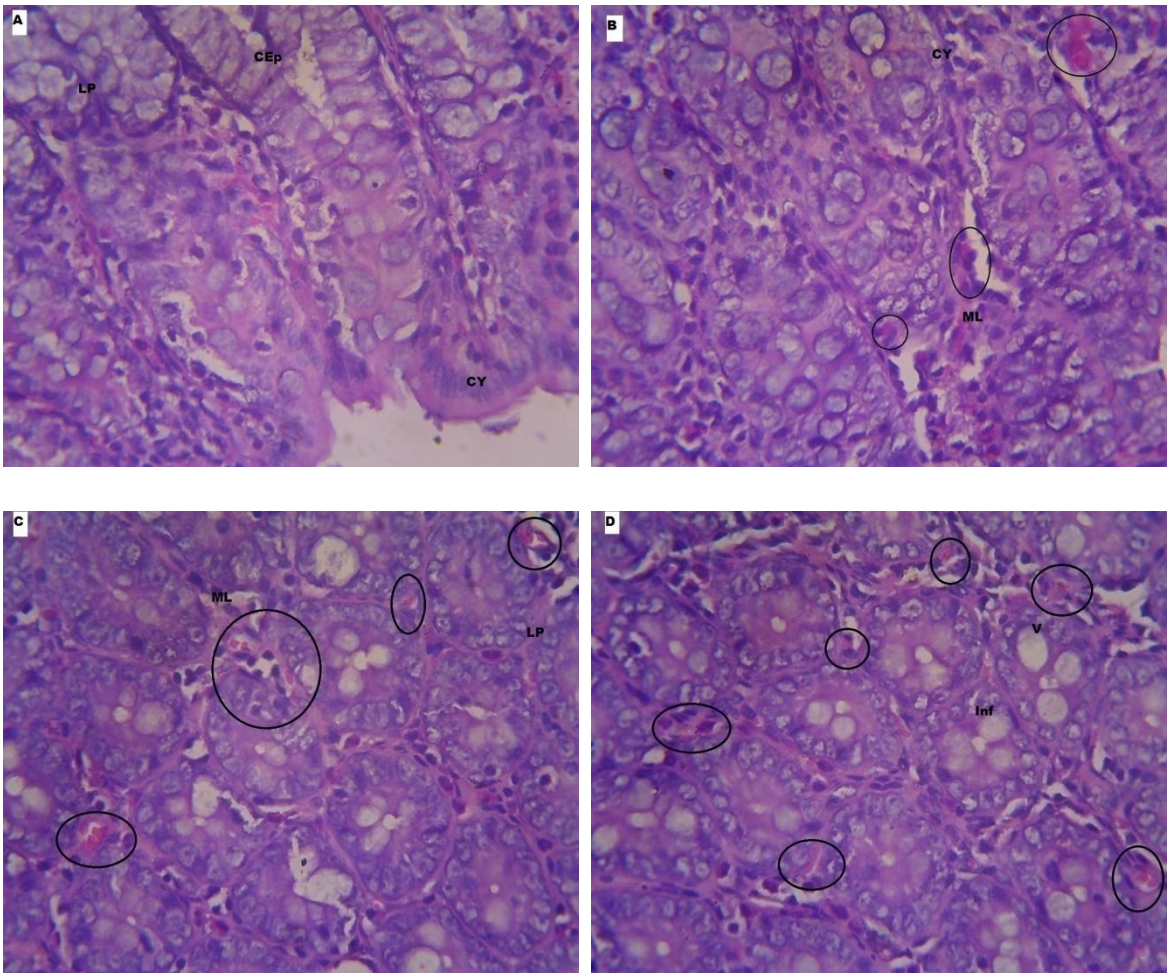
n=6, DAI: disease activity index, BSCB: *Bacillus coagulans* bacterial probiotics.

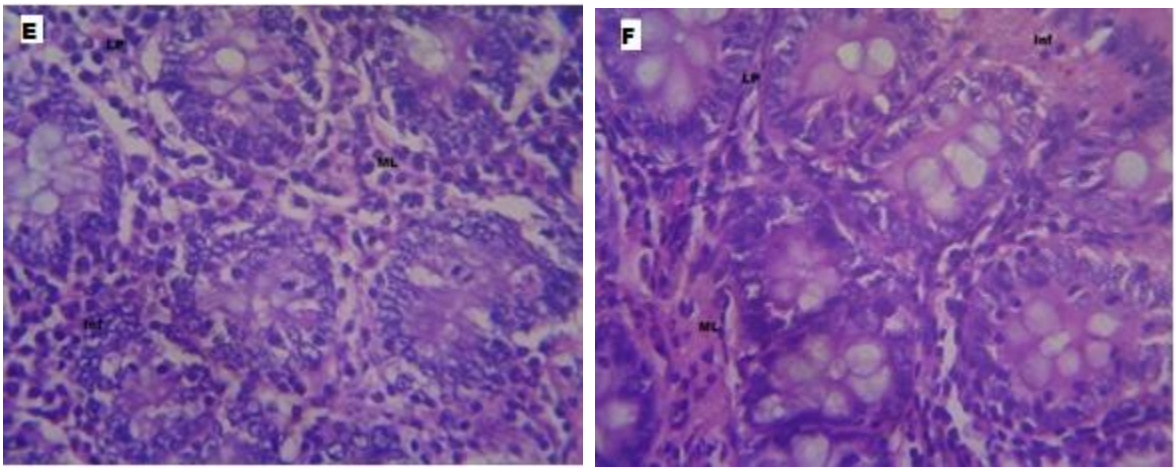
Superscript letters denoted the Duncan homogenous post hoc testing between the tested groups.

3.2. Histological analysis

Group I: Control colon tissues showed proper arrangement of lumen crypts (CY) which contains columnar epithelial cells (CEp). Histological sections showed lamina propria (LP) surrounded by mucosal layer (ML) with infiltrated macrophages and lymphocytes (Figure 2A & 2B). Group II: Oral administration of DSS showed damaged crypts with reduced mucosal layers. Increased vacuolation (V) in the lamina propria (LP) regions with increased with infiltrated (Inf) eosinophil and lymphocytes (Figure 2C & 2D). Group III: BSCB-2 Probiotics administration in DSS colitis induced rats showed mucosal layer (ML) rearrangement. Crypts were reformed with properly arranged lamina propria (LP) with infiltrated lymphocytes (Figure 2E & 2F).

Figure 2. Male albino wistar rat colon histological sections (40×) of control, DSS colitis induced and DSS + *B. coagulans* BCSB-2 treated groups





4. Discussion

Lee *et al.* (2009) reported the strong correlation between the DAI with colon histology and also concluded that improved intestinal matrix enhances the colon degenerated tissues in colitis induced groups. Mao *et al*. (2016) reported the effect of QCWZD, a Chinese traditional medicine against DSS colitis induced in rats. 4.5% DSS were used to induce colitis in rats. At the end of experimental duration (7days), DAI and colon histology were studied. When compared to DSS colitis rats, QCWZD treated rat groups showed decreased DAI score and improved colon histology. Solomon *et al.* (2010) reported weight loss, rectal bleeding and bloody stool diarrhea in DSS colitis induced rats.

Wang *et al*. (2017) reported DSS induced colitis rat system showed significantly ($P<0.001$) increased DAI score than control group rats. The remission of UC in male rats after standard drug treatments, whereas significantly alleviated DAI in colitis induced rat and reduced DAI after treatment. Acute DSS (3%) colitis induced female mice colon showed typical histological changes such as epithelial degeneration, crypt abscesses, mucin depletion, cryptitis, neutrophils infiltrations in lamina propria and submucosa layers and necrosis condition in intestinal epithelial cells, vacuolar hydropic degeneration of intestinal epithelial cells (Perse and Cerar 2012).

DSS exposure affected DNA synthesis in columnar epithelial cells resulted in lack of proliferation and reduced mucosal synthesis in colitis induced mice (Araki *et al.*, 2010). 5% DSS induced male wistar rats showed significantly increased DAI with increased colon tissue damages than compared to control group rats (Oliveira *et al.*, 2014). An infiltrated immune cell at the reaction site was the primary response of inflammation (Ostvik *et al.*, 2013). Similar results observed in DSS colitis induced rat colon tissues. Prokopowicz *et al.* (2012)

reported infiltrated cells, inflammated lamina propria with degenerated crypts with reduced mucous secreting goblet cells in colitis induced rat colon tissues.

Histological colon architecture revealed the infiltration of lymphocytes and neutrophils into the colon mucosal layers of colitis induced rats which resulted in the increased production of neutrophils and lymphocytes in rat system (Rajendiran et al. 2018). DSS induced colitis model showed submucosal erosions in colon regions, cell infiltrations with inflammation, ulcerations, epithelioglandular hyperplasia with crypt abscesses (Uko *et al.*, 2012) and immune cells activation in lamina propria in the epithelial regions of colon (Wirtz and Neurath, 2007).

Conclusion

Effects of orally administrated BSCB-2 probiotics in colitis induced male albino wistar rats were studied. DSS induced the colitis disease in male rats which showed degenerated colon tissues resulted in increased DAI and gross rectal bleeding whereas *Bacillus coagulans* probiotics administration relapsing the normal histology of colon resulted in significantly reduced DAI and rectal bleeding. Our study concluded that BSCB-2 probiotics cure and retain the colon histoarchitecture in DSS colitis induced male rats.

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