Exploring Probiotic Mediated Amelioration of Inflammation in DSS Induced Colitis Rat

Manisha Kumari, Anu Kumari, Dolly Sharma, Harshita Gupta and Ramesh C. Meena

Department of Pathophysiology and Disruptive Technologies

Defence Institute of Physiology and Allied Sciences, Timarpur, Delhi-110054, India.

Email:prajapati.manisha161@gmail.com

INTRODUCTION

- Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by persistent inflammation and damage to the colon's inner lining.
- Its complex development involves genetics, environment, gut microbes, and immune system [2].
- Conventional therapies for the treatment of UC, focus mainly on managing symptoms via pharmacotherapy, with or without surgical intervention but issues like drug intolerance and long-term dependence exist.
- Nutritional supplementation has been known to play a decisive role in ameliorating UC.

OBJECTIVE

To examine the prophylactic potential of a probiotic cocktail containing *Lactobacillus sp.*, *Bifidobacterium sp.*, *Bacillus sp.*, and *Saccharomyces sp.*, in amelioration of intestinal inflammation and gut dysbiosis in colitis.

METHODOLOGY

- A commercially available composition enriched with *Lactobacillus acidophilus* (1.6 x 10^9 CFU/g), *Lactobacillus rhamnosus* (0.8 x 10^9 CFU/g), *Bifidobacterium longum* (0.8x10^9 CFU/g), *Saccharomyces boulardii* (0.2x10^9 CFU/g), *Bacillus coagulans* (1.6 x 10^9 CFU/g) was dissolved in 2ml drinking water, supplemented orally to animals.
- 40 healthy male *Sprague dawley* rats were divided into four groups namely: Control (drinking water), PB (prophylactic administration of oral probiotic cocktail in water), DSS (5% DSS in water to induce colitis for 9 days), and D-PB (prophylactic administration of oral probiotic cocktail starting 4 days prior to 5% DSS in water for 9 days).
- After 9 days of intervention, the disease activity index (DAI) was assessed.
- The histo-pathological alterations and mucus-containing goblet cells were visualized using haematoxylin-eosin. The levels of lactoferrin and lipocalin-2 were measured in fecal pellets and the expression levels of pro and anti-inflammatory cytokines were measured in serum.
- 16S rRNA was performed in the colonic content to investigate the regulatory effect of probiotic on microbial structure and functional potential.

RESULTS

Impact of DSS and probiotic administration on large intestine

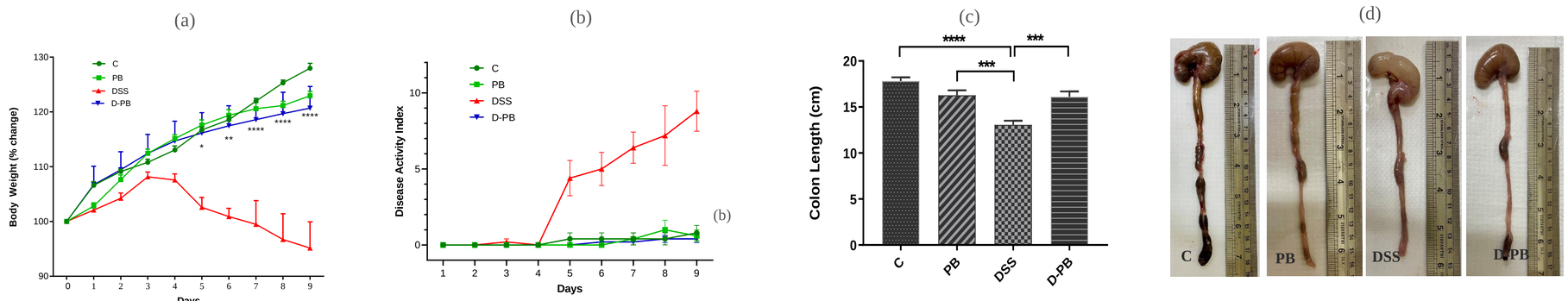

Fig. 1: Effect of Probiotic on DSS induced colitis: (a) Percentage weight change; (b) Disease Activity Index; (c) Colon Length (g); (d) Representative images of rat colon length. (n=10). Data shown as mean± SEM.. Level of significance is denoted with six teardrop spoked propeller asterisk with p-value <0.05="", p < 0.01="**", p < 0.001 ="***", p < 0.0001= "****"*

Assessment of Intestinal inflammation markers in fecal supernatants and Pro & Anti-inflammatory markers in serum

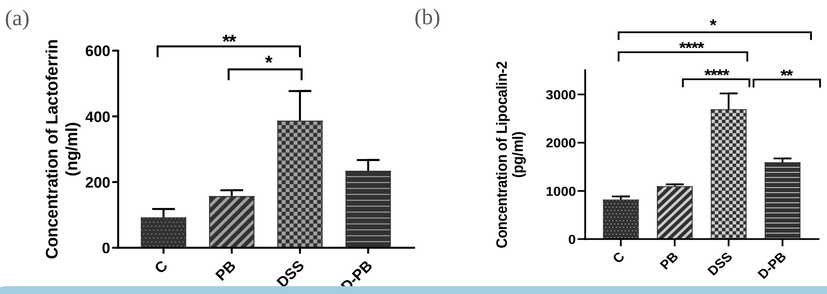

Fig. 2: Evaluation of intestinal inflammation markers (a) Lactoferrin and (b) Lipocalin-2 in Fecal supernatants (n=5). Data shown as mean± SEM.. Level of significance is denoted with six teardrop spoked propeller asterisk with p-value <0.05="", p < 0.01="**", p < 0.001 ="***", p < 0.0001= "****"*

Estimation of Pro and Anti-inflammatory markers in serum

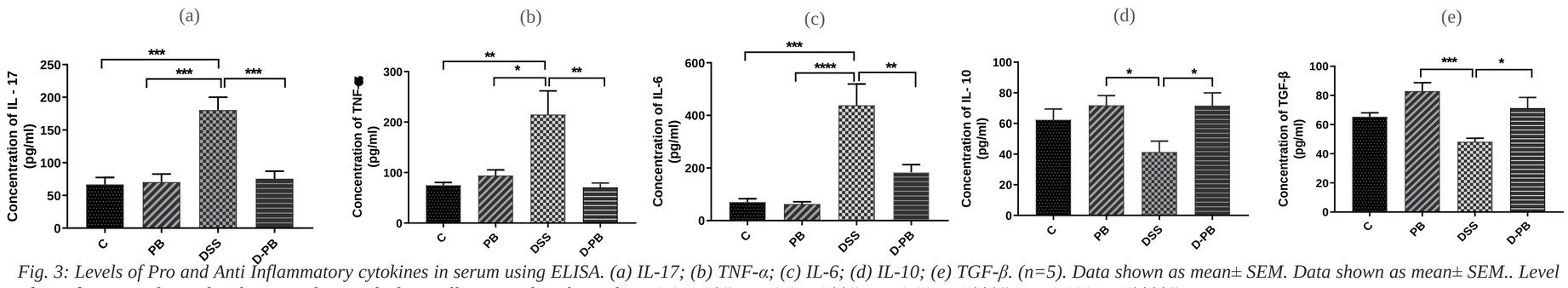

Fig. 3: Levels of Pro and Anti Inflammatory cytokines in serum using ELISA. (a) IL-17; (b) TNF-α; (c) IL-6; (d) IL-10; (e) TGF-β. (n=5). Data shown as mean± SEM. Data shown as mean± SEM.. Level of significance is denoted with six teardrop spoked propeller asterisk with p-value <0.05="", p < 0.01="**", p < 0.001 ="***", p < 0.0001= "****"*

Structural alterations: Histological analysis by HE staining

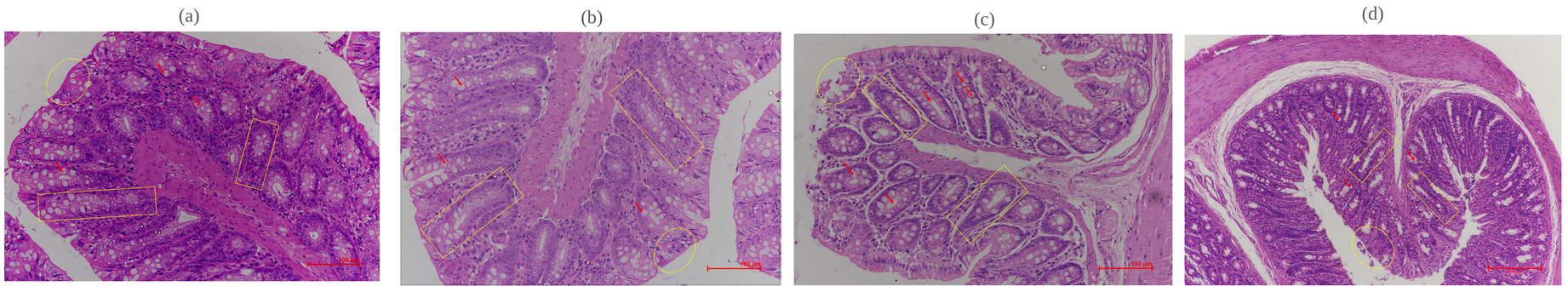

Fig. 4: Epithelial damage and crypt lesions in distal colon from different groups were evaluated by H&E staining. (a) C: Control, (b) PB: Probiotic group, (c) DSS: DSS group, (d) D-PB group: DSS along with probiotic administration group. In these HE stained pictures scale bar is mentioned in μm at right bottom corner, Yellow box indicates Crypt, Yellow circle indicates epithelial lining and red arrow shows goblets cells.

Analysis of Gut microbiota composition and functional potential

Fig. 5: Metagenomic insights into rat gut microbiota compostition, diversity and functional dynamics after DSS and probiotic administration. (a) Composition of rat gut microbiome (b) Alpha and beta diversity analysis (c) Predicted functional potential of microbial communities.

KEY FINDINGS

- Prophylactic probiotic administration alleviated colonic inflammation by significantly decreasing the DAI scores. In addition, the change in colon shortening in the DSS group was reversed by probiotic intervention.
- Lactoferrin and Lipocalin-2 are fecal biomarkers which are used to assess intestinal inflammation. Probiotic administration has been shown to decrease their expression in fecal supernatant. Furthermore, prophylactic probiotic administration shows an anti-colitis effect by significantly inhibiting the sécrétion of pro-inflammatory cytokines in the D-PB group.
- The DSS group exhibited injury to superficial epithelium layer, dilated cryptic structures, and fewer goblet cells. Administration of probiotic revealed a largely preserved mucosal lining with insignificant inflammation, non-dilated and non-necrotic crypts, and intact goblet cells in the D-PB group.
- Probiotic administeration could alleviate the clinical symptoms of IBD in rats and achieved a mitigatory effect by regulating the expression of cytokines, restoration of gut microbial structure.

CONCLUSION

- Taken together, the probiotic cocktail effectively alleviated intestinal inflammation by modulating immune system, mucosal inflammation and enhancing intestinal barrier functions, suggesting its great potential to be a novel therapeutic approach for the treatment of UC.