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P. pentosaceus Administration Attenuates the Severity of Dextran Sulfate Sodium-Induced Colitis and Improve the Intestinal Permeability

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LTA, LRF and JRE designed the study, performed the statistical analysis, wrote the protocol, managed literatures researches, managed the analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate the effect of different doses of *P. pentosaceus* on colitis symptoms as well as in the intestinal barrier function in an *in vivo* model of acute ulcerative colitis induced with dextran sodium sulfate (DSS).
Study Design: Experimental.
Place and Duration of Study: Laboratory of Pharmacology, Toxicology and Immunomodulators, Facultad de Farmacia, Universidad Autonoma del Estado de Morelos, Mexico, between march 2015 and December 2015.
Methodology: Acute colitis was induced in BALB/c mice via the administration of 3.5% DSS in drinking water administered ad libitum for 7 days. On day 2 during the induction of colitis, four groups received different doses of *Pediococcus pentosaceus* (1×10^7, 1×10^8, 1×10^9, and 1×10^{10} Colony Forming Units (CFUs)) via gastric tube once per day. We assessed the severity of colitis

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based on disease activity index (DAI), colon length and histological damage; colonic permeability to fluorescein isothiocyanate–dextran (FITC-dextran) was measured *in vivo.*
Results: Treatment with *P. pentosaceus* at doses of $1x10^{10}$ significantly reduced the DAI compared with the DSS group (1.94± 0.93 vs. 3.54± 0.61) and decreased the diarrhea score and fecal blood score (1.53 ± 0.35 vs. 3.73). *P. pentosaceus* improved colon length (8.81 ± 0.95 cm vs. 7.26 ± 0.67 cm) and decreased intestinal permeability by 49.6% compared to the DSS group.
Conclusion: The administration of *P. pentosaceus* at a dosage of $1x10^{10}$ CFUs attenuates the severity of DSS-induced colitis and improves epithelial barrier function.

Keywords: Ulcerative colitis; Pediococcus pentosaceus; probiotics; barrier function; permeability.

1. INTRODUCTION

Ulcerative colitis (UC) is a chronic disease characterized by diffuse inflammation of the mucosa of the colon and rectum. Its classic clinical symptom is bloody diarrhea, and its clinical course is characterized by periods of remission and exacerbation, which may occur either spontaneously or in response to treatment changes or intercurrent illnesses [1]. UC worldwide occurrence has increased over the past few years, and publications show an upsurge in developing countries across Latin America, Asia and Eastern Europe [2].

Extensive research has tried to determine pathogenic mechanisms and develop efficacious therapies. Four fundamental components underlying UC pathogenesis have been identified, and these represent likely sources for the yet undefined etiological factors: environment, microbiota, immune system, and genome [3-4]. While these studies have helped define the clinical course of UC, a safe and effective treatment remains elusive.

There is abundant evidence that commensal bacteria are involved in the pathogenesis of human ulcerative colitis as well as experimental colitis [5]. Many genetically susceptible models do not develop colitis when raised in a germ-free environment. In fact, disease in most models can be attenuated or completely abolished with antibiotic treatment [6-7].

Although no microorganism is directly associated with UC, there is a link between UC and profound changes in the diversity and composition of the microbiota [8]. The fecal microbiota of inflammatory bowel disease patients show a decrease in the frequency of the phyla *Bacteroidete*s and *Firmicutes* and an increase of *Proteobacteria* and *Actinobacteria* [9].

More specifically, UC patients subjected to molecular detection methods, but not controls, showed a greater prevalence of *Campylobacter spp*., *Enterobacteriae*, and enterohepatic *Helicobacter*. Additionally, serologic testing identified *Fusobacterim varium* as a potential contributor to intestinal inflammation in UC. Interestingly, *in-situ* hybridization studies have shown that anti-inflammatory *Lactobacillus* spp. and *Pediococcus* spp. were absent in samples from subjects affected by UC [10].

Consequently, interest in the therapeutic potential of microbiota modifications has increased. Probiotics are live microorganisms that, when administered in adequate amounts, can confer health benefits to the host [11] and are considered a promising alternative therapy for UC. To date, *Lactobacilli, Bifidobacteria* [12], VSL#3 (a compound probiotic preparation composed of four *Lactobacilli* strains, three *Bifidobacteria* strains, and one *Streptococcus salivarius* strain) [13], and *Escherichia coli* (*E. coli* Nissle 1917 have been applied to UC subjects both animal models and human cases and effectively resolved disease symptoms). The degree to which probiotics can successfully resolve UC varies depending on the strain, dosage and time of administration, which has led to research on new bacteria with probiotic potential.

DSS-induced colitis in rodents is characterized by epithelial disruption, resulting in luminal bacterial translocation and subsequent infiltration of neutrophils and other acute immune cells. These features recapitulate the events that lead to the acute mucosal injury of human ulcerative colitis [14].

Many strains of lactic acid bacteria (LAB) are typically regarded as safe because of their long history of use, and their status is generally recognized as safe. In addition to demonstrating

the efficacy of probiotics in improving human health, safety characteristics must be considered. *Pediococcus pentosaceus* is a crucial industrial starter culture for food fermentation, such as various meats, vegetables, and cheeses [15]. There is evidence that *P. pentosaceus* possess probiotic properties [16-18]. However, these have yet to be assessed in the DSS-induced colitis model. The aim of this study is to evaluate the effect of different doses of *P. pentosaceus* on symptoms of colitis as well as in the intestinal barrier function in an *in vivo* model of acute colitis induced with dextran sodium sulfate.

2. MATERIALS AND METHODS

Eight-weeks-old male BALB/c mice were purchased from Harlan Laboratories, Mexico. The mice were allowed to adapt to their environment for 1 week, and kept in an environmentally controlled room with 12 h light-dark cycle at 25±1°C. All procedures were approved by the Institutional Animal Care and Use Committee of the Veterinary Medical School at the National Autonomous University of Mexico. Experiments were conducted following the rules and principles set in the Guide for the Care and Use of Laboratory Animals.

2.1 Bacterial Strain and Culturing Conditions

Pediococcus pentosaceus were cultured anaerobically in MRS broth at 37ºC for eight hours. The bacteria were harvested by centrifugation at 4°C, 5000 g for 10 min, washed in 0.89% of salin solution, resuspended in 10% of fat-free milk and was freeze-dried and stored at 4°C prior to use.

2.2 Experimental Design

2.2.1 Pediococcus pentosaceus in healthy mice

Mice were divided into five groups (n=6/group): Control, *P. pentosaceus* ($1x10^4$), *P. pentosaceus* ($1x10^6$), *P. pentosaceus* ($1x10^8$), *P. pentosaceus* ($1x10^{10}$). *P. pentosaceus* was administered daily at different doses for 5 days ($1x10^4$, $1x10^6$, $1x10^8$ and $1x10^{10}$ CFUs). During the study, the weight, stool consistency and any hemorrhaging were measured; the microscopic morphology of the colon was monitored.

Acute colitis was induced by DSS treatment using a method previously described by others [19]. DSS was administered in the drinking water (3.5% w/v) for 7 days (DSS, molecular weight 40 kDa; Sigma Aldrich). The mice were then randomly divided into the following control and experimental model groups (n = 6 each one): negative-treatment model DSS (administered gavage of PBS); experimental-treatment models (administered gavage of 10^7, 10^8, 10^9, or 10^{10} CFU/100 μl of *P. pentosaceus*, respectively). In the four DSS-induced colitis groups, *P. pentosaceus* was administered in different doses ($1X10^7$, $1x10^8$, $1x10^9$ or $1x10^{10}$ CFUs respectively) by oral gavage, in sterile PBS and along with DSS, from day two until day six (*P. pentosaceus* +DSS). The colitis disease activity index (DAI) was determined based on previous studies of DSS-induced colitis [20]. No weight loss was counted as 0 points, weight loss of 1 to 5% as 1 point, 5 to 10% as 2 points, 10 to 20% as 3 points, and >20% as 4 points. For stool consistency, 0 points were given for well formed pellets, 2 points for pasty and semiformed stools that did not stick to the anus, and 4 points for liquid stools that did stick to the anus. Bleeding was scored 0 points for no blood in stool, 2 points for blood in stool, and 4 points for gross bleeding. These scores were added and divided by 3, forming a total clinical score that ranged from 0.0 (healthy) to 4.0 (maximal activity of colitis).

2.3 Permeability Essay

Twenty-one male BALB/c mice weighing 20–22 g were divided into three groups: the Control, Colitis and Colitis+ *P. pentosaceus* groups (n = 7 each). Acute colitis was induced by administering dextran sodium sulfate (DSS; molecular weight 40 kDa) 3.5% wt/vol in drinking water ad libitum for 6 days. On day 2 during colitis induction, the mice were intragastrically gavaged with *P. pentosaceus* (doses $1x10^{10}$ Colony Forming Units). On the 7th day of the study, the intestinal permeability was measured via the determination of the fluorescence intensity of FITC-dextran (molecular weight 4.0 kDa, Sigma Aldrich- FD4) in serum after 4 hours of oral administration by spectrophotofluorometry with an excitation of 485 nm and an emission wavelength of 528 nm [21].

2.4 Histologic Analysis

Histological examination was performed for each animal on three samples of the distal colon; the tissues were fixed and embedded in paraffin using standard procedures, and the sections (5 μm) so obtained were stained with hematoxylin and eosin. Colon tissues were examined and

imaged using a Olimpus IX81 microscope equipped with an camera.

All statistical analyses were carried out with the Graphpad software suite (version 16.0). Results are expressed as mean ± SEM. If the variance was homogeneous, single-factor analysis of variance (one-way ANOVA) was used to analyze the differences between groups.

3. RESULTS AND DISCUSSION

Healthy mice treated with different doses of *P. pentosaceus* ($1x10^4$, $1x10^6$, $1x10^8$, and $1x10^{10}$ UFCs) showed no significant differences in the parameters evaluated (i.e., weight loss, colon length, changes in the consistency of feces with regards to the control group; Table 1). Similarly, no changes were identified in the colon histologies of *P. pentosaceus* pertaining to different doses. Lieberkühn crypts, goblet cells and enterocytes showed normal architecture and morphology when compared with the control group (Fig. 1).

In contrast to untreated control mice, mice exposed to DSS developed symptoms of acute colitis with diarrhea, rectal bleeding, wasting, and

Table 1. Effects of different doses of *P. pentosaceus* treatment on weight, colon length, stool consistency and fecal blood in healthy BALB/c mice

	P. pentosaceus				
	Control	**$1x10^4$**	**$1x10^6$**	**$1x10^8$**	**$1x10^{10}$**
Weight (g)	23.86 ± 0.79	23.44 ± 0.76	23.26 ± 1.14	22.92 ± 1.18	22.12 ± 0.97
Colonic length (cm)	11.28 ± 1.14	10.02 ± 0.45	10.04 ± 0.30	10.48 ± 0.30	10.36 ± 0.69
Stool consistency	Normal	Normal	Normal	Normal	Normal
Fecal blood	0	0	0	0	0

*Weight and colon length they are expressed as mean ± SEM; Stool consistency and Fecal blood data are expressed as median score (range). * Indicates a significant difference compared to p<0.05 compared to control (n=6)*

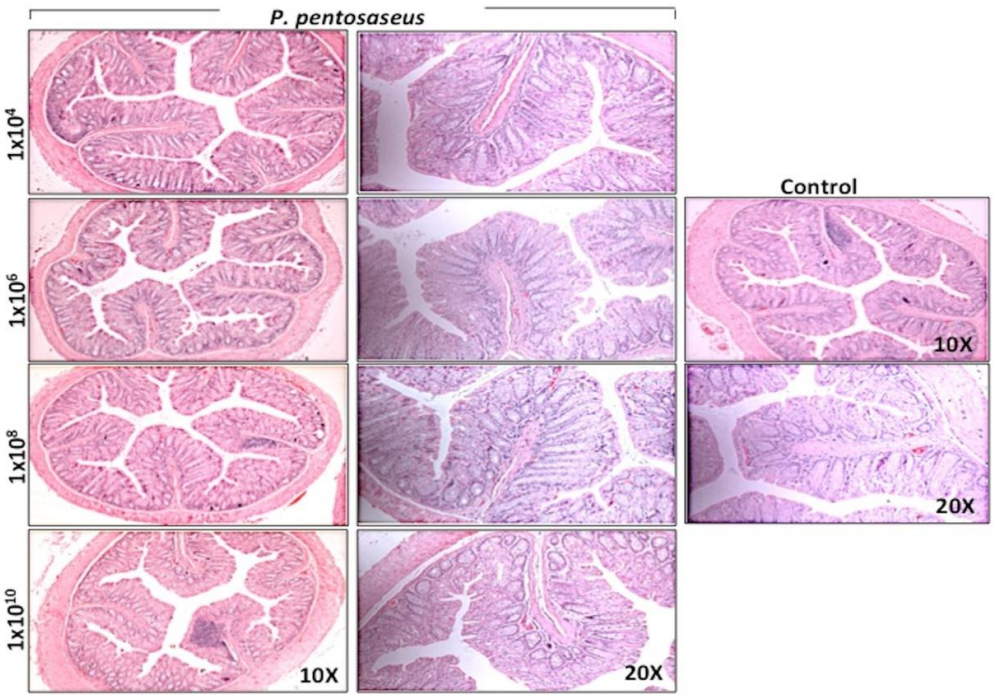

Fig. 1. Effect of different doses of *P. pentosaceus* treatment on the histological morphology of the colon of BALB/c mice

Representative photomicrographs stained with hematoxylin and eosin of the distal colon of five mice, each belonging to a different P. pentosaceus dosage group plus control group following 5-day treatment. The doses per group were $1x10^4$, $1x10^6$, $1x10^8$, and $1x10^{10}$ CFUs (magnification 10x and 20x)

a loss of body weight. From the fourth day on, all groups exhibited a significantly higher DAI score during DSS treatment when compared to the healthy control group ($p < 0.05$; Fig. 2). The results indicated no significant DAI difference in the DSS+ *P. pentosaceus* $1x10^7$, DSS+ *P. pentosaceus* $1x10^8$, and DSS+*P. pentosaceus* $1x10^9$ groups when compared with the DSS group. However, the DAI scores of animals in the DSS+*P. pentosaceus* $1x10^{10}$ group were significantly less than those of the DSS group (1.94± 0.93 vs. 3.54± 0.61, $p < 0.05$) on the last day of the study. This decrease in the DAI was due to a reduction in the diarrhea score in the DSS+*P. pentosaceus* $1x10^{10}$ group with respect to the DSS group (1.53 ± 0.35 vs. 3.73, $p<0.05$; Fig. 3), and a decrease in the fecal blood score starting on the 5th day of the study when compared to the DSS group (1.25 ± 0.39 vs. 3.85; Fig. 4). We observed no differences in body weight (data no shown).

The DSS-induced model of colitis is associated with a significant decrease in colon length [22]. All groups exhibited significant colon length reduction during DSS treatment when compared to the healthy control group ($p<0.05$). However, the DSS+ *P. pentosaceus* $1x10^{10}$ group had significantly improved upon colon length shortening when compared with the DSS group. (8.81 ± 0.95 cm vs. 7.26 ± 0.67 cm, $p< 0.05$; Fig. 5).

We analyzed the intestinal permeability to FITC-dextran, a fluorescein labelled dextran, in mice with colitis who were treated with *P. pentosaceus at doses of* $1x10^{10}$ *UFC.* The DSS group showed higher plasma levels of FITC-dextran 4 h after oral administration of FITC-dextran than the control group did, indicating the disruption of the intestinal barrier by DSS. At the same point, the plasma FITC-dextran levels in the *P. pentosaceus* + DSS group *was* lower than in the DSS group ($p<0.05$), and the level was approximately equal to that in the control group (Fig. 6). Thus, *P. pentosaceus* decreased the intestinal permeability caused by DSS.

Once it was observed that treatment with *P. pentosaceus* delayed some symptoms of ulcerative colitis, we wondered whether *P. pentosaceus* affected colon morphology in an acute colitis model (Fig. 7). The mice in the control group showed a normal, orderly arrangement of the cellular structure of colonic tissue, with no perturbations in goblet cell number or mucosal integrity. In contrast, the DSS group presented changes: histological findings in colon tissue showed superficial inflammation mainly affecting the mucosa, as well as a loss of goblet cells; distortion of the crypts followed by

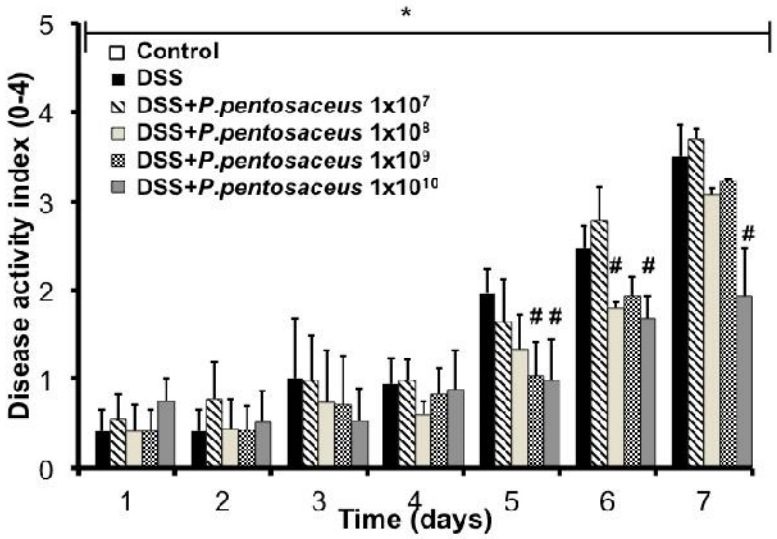

Fig. 2. Effect of different doses of *P. pentosaceus* treatment ($1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ CFUs) on the disease activity index in DSS-induced colitis in BALB/c mice

*The treatment with P. pentosaceus was administered on second day of DSS-induced colitis. Data are expressed as mean ± SEM. * mean value was significantly different from that of the Control group ($p<0.05$), # mean value was significantly different from that of the DSS group ($p<0.05$) (n=6)*

shortening of the same, and infiltration of inflammatory cells in the mucosa and submucosa. *P. pentosaceus* at the doses ($1x10^8$, $1x10^9$ and $1x10^{10}$ CFUs) showed a loss of crypt architecture and goblet cells in the mucosa accompanied by a cellular infiltrate, however, in comparison to DSS group, these groups did not show an increase in the size of the submucosa the damage only extended in the mucosal area and muscularis mucosae.

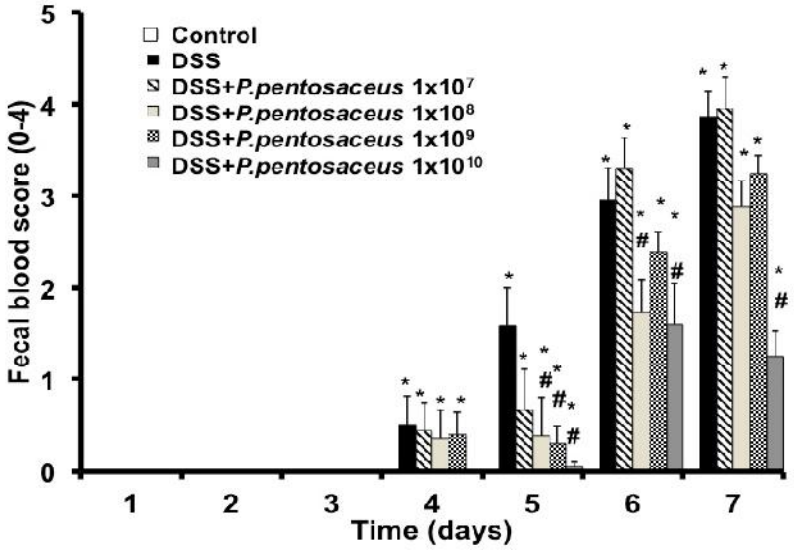

Fig. 3. Effect of different doses of *P. pentosaceus* ($1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ CFUs) on the diarrhea score of DSS-induced colitis in BALB/c mice

*The treatment with P. pentosaceus was administered on second day of DSS-induced colitis. Data are expressed as mean ± SEM. * mean value was significantly different from that of the Control group (p<0.05), # mean value was significantly different from that of the DSS group (p<0.05) (n=6)*

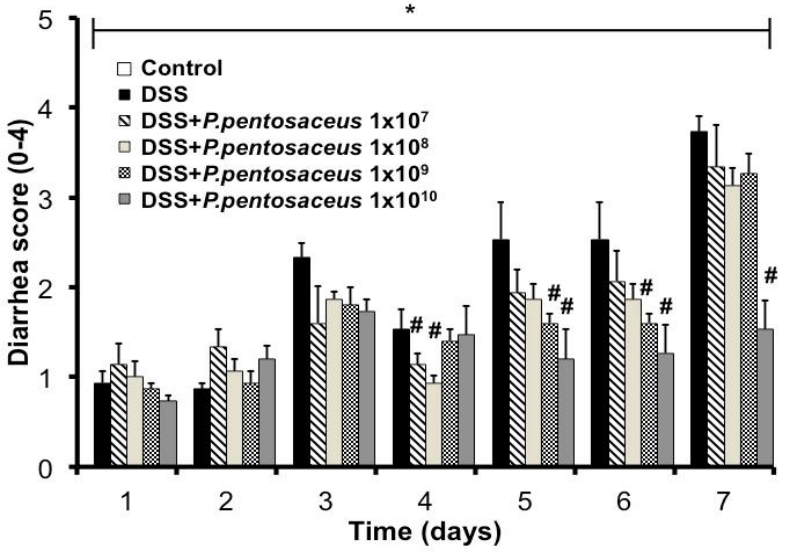

Fig. 4. Effect of different doses of *P. pentosaceus* ($1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ CFUs) on fecal blood in DSS-induced colitis in BALB/c mice

*The treatment with P. pentosaceus was administered on second day of DSS-induced colitis. Data are expressed as mean ± SEM. * mean value was significantly different from that of the Control group (p<0.05), # mean value was significantly different from that of the DSS group (p<0.05) (n=6)*

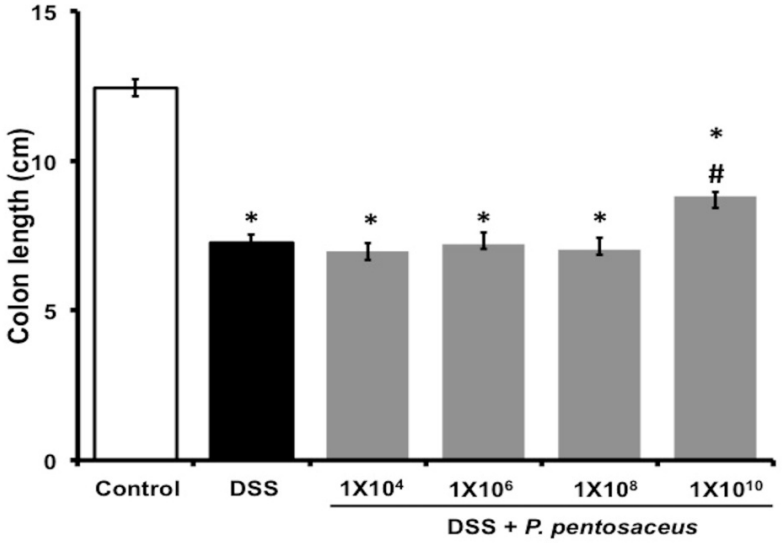

Fig. 5. Effect of different doses of *P. pentosaceus* ($1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ CFUs) on colonic length in DSS-induced colitis in BALB/c mice

*The treatment with P. pentosaceus was administered on second day of DSS-induced colitis. Data are expressed as mean ± SEM. * mean value was significantly different from that of the Control group (p<0.05), # mean value was significantly different from that of the DSS group (p<0.05) (n=6)*

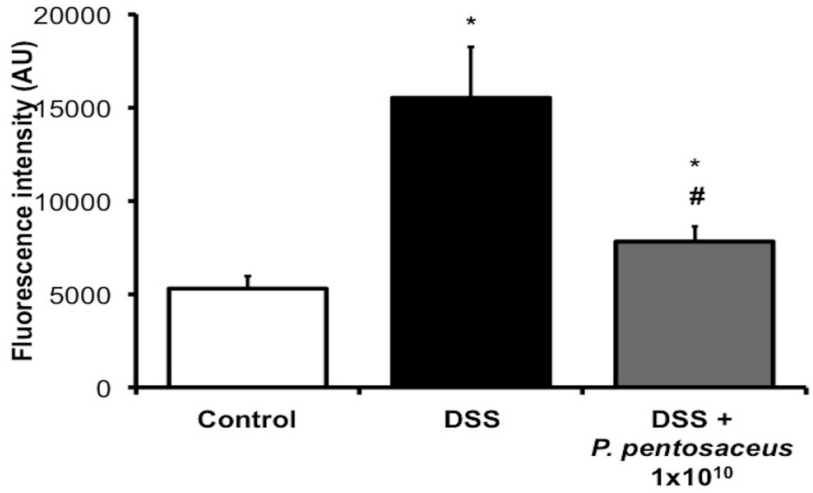

Fig. 6. Effect of *P. pentosaceus* (doses $1x10^{10}$ UFC) on intestinal permeability in DSS-induced colitis in BALB/c mice

*Plasma fluorescence intensity of FITC-dextran levels were measured 4h after oral administration of FITC-dextran in each group. Data are expressed as mean ± SEM. * mean value was significantly different from that of the Control group (p<0.05), # mean value was significantly different from that of the DSS group (p<0.05) (n=7)*

3.1 Discussion

This study evaluated the effect of a probiotic bacterial species as a potential candidate in the treatment to ease symptoms of DSS-induced colitis in mice.

The main symptoms of DSS-induced colitis are diarrhea, rectal bleeding, and weight loss. Inflammation also entails severe lesions along the mucosa, alteration of the epithelial structure, extensive infiltration of neutrophils and lymphocytes into the mucosa and sub-mucosa, and crypt loss [22].

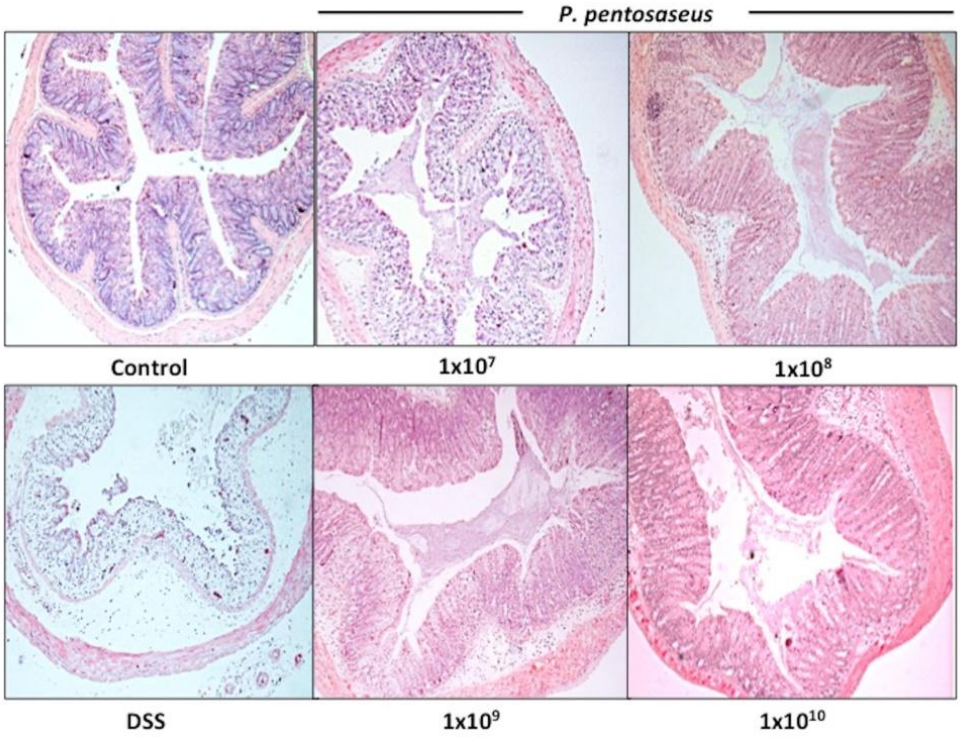

Fig. 7. Effect of different doses of *P. pentosaceus* (1x10^7, 1x10^8, 1x10^9 and 1x10^{10} CFUs) on the histological morphology of the colon of BALB/c mice with colitis induced-DSS

The treatment with P. pentosaceus was administered on second day of DSS-induced colitis. Stained with Hematoxylin and Eosin (magnification 10x)

Several experimental models of colitis show probiotics have therapeutic and prophylactic effects [23-25]. According to these studies, the administration of probiotic bacteria can improve the symptomatology and pathology in animals; the most frequently used bacterial species are *Lactobacillus* and *Bifidobacterium*. Mixtures of bacterial strains, such as probiotic VSL # 3, have also been used for the same purpose [26]. Although no microorganism is directly associated with ulcerative colitis, dysbiosis has been found in both patients and *in vivo* models of colitis. Specifically, studies on patients have shown a decrease in the Lactobacillus and Pediococcus species [10].

The probiotic effect of certain strains of *P. pentosaceus* has demonstrated immuno-modulatory effects [27-29]. However, these have not been tested on an acute DSS-induced colitis model, which this study addresses.

Acute sub-chronic and chronic toxicity studies have been performed on some currently employed probiotics and no adverse effects have been found. This includes high oral dosages and consistent use for long periods of time [30-34]. Since not all the probiotics have the same therapeutic characteristics, we decided to evaluate the effect of different oral doses of *P. pentosaceus* (1x10^4, 1x10^6, 1x10^8 and 1x10^{10} UFCs). As expected, administration of the bacteria did not modify body weight, stool consistency or the microscopic structure of the colon, so we concluded *P. pentosaceus* is safe and could be employed as a possible treatment in a model of colitis.

One of the aspects to consider regarding the study of probiotics is the dose at which it is to be evaluated. It has been reported that the effect of a microorganism can vary depending on the administered dose. For example, the administration of different doses of Lactobacillus acidophilus (10^4, 10^5, 10^6, 10^7 and 10^8 CFUs / 10g) in a murine model of DSS-induced acute colitis decreased disease activity index, protected against weight loss and the shortening of the colon. However, the dose with the greatest effect in this model was 1x10^6 CFUs / 10 g [35].

Hence the importance of evaluating the effect of *P. pentosaceus* at different doses in a model of DSS-induced ulcerative colitis.

Our study shows that the effect of *P. pentosaceus* is dose dependent. A dose of $1x10^{10}$ CFUs was able to reduce the disease activity index, delaying the onset of diarrhea and hemorrhage; lower doses had no visible effect. Also, the dose of $1x10^{10}$ CFUs helped prevent the colon shortening associated with DSS, decreased intestinal permeability to FITC-dextran, and structurally improved the microscopic architecture of the colon. Although the $1x10^{10}$ dose of *P. pentosaceus* had beneficial effects in animals with DSS-induced colitis, it did not change the weight of these group. Other studies, such as Izumi et al, have obtained similar results following the administration of *bifidobacterium* [36]. Further studies must quantify food consumption. If this is being modified, this would indicate a possible metabolic effect.

Future studies need to increase the *P. pentosaceus* dose to identify the optimal, most effective dose in this model. The results show that *Pediococcus pentosaceus* is capable of decreasing UC activity as well as anatomopathological alterations.

The DSS-induced colitis model is the most widely used for *in vivo* studies and allows us to evaluate various aspects of mucosal barrier integrity as well as its function [22]. Microscopically it was observed that the treatment with *P. pentosaceus* partially restored the architecture of the distal colon; areas of the epithelium preserved the distribution of well-defined Lieberkühn crypts. This effect was observed in doses of $1x10^{8}$, $1x10^{9}$ $1x10^{10}$.

We wondered what the mechanism of action would be, since probiotics exert their effect via different ones. First, they act as a barrier via competitive inhibition, which prevents other pathogenic bacteria from reaching the lamina propria and stimulating the immune system of the mucosa. Second, probiotics increase the production and thickness of mucus, which protects against invasive bacteria, and probiotics can alter the consistency of mucus, thus changing the patterns of bacterial adhesion. Third, probiotics modulate the immune system by exerting an anti-inflammatory and less pro-inflammatory effect, as well as secreting protective immunoglobulins like IgA [37].

Studies on patients with ulcerative colitis and experimental models of colitis have shown an increase in intestinal permeability due to the decrease in the expression and distribution of the proteins of tight junctions like ZO-1 and Occludin. After observing *P. pentosaceus* improved the symptoms of colitis at a dosage of $1x10^{10}$ UCFs, we decided to evaluate its effect on the intestinal epithelial barrier function, where a decrease in intestinal permeability to FITC-dextran was observed in the DSS-induced model of colitis. This indicates that *P. pentosaceus* is increasing the intestinal barrier function. In a murine model of DSS-induced colitis, oral administration of VSL # 3 probiotic improved intestinal barrier function because decreased the intestinal permeability by maintaining tight junction protein expression and preventing apoptosis [38], the probiotic VSL # 3 consists of a set of 8 strains that acted synergistically to exert the effect of intestinal epithelial barrier protection. Our probiotic P. pentosaceus was able to modify this function, showing a similar effect to decrease the permeability to FITC / dextran, so it is necessary to study if it has an effect on the expression and distribution of the tight junction proteins.

4. CONCLUSION

In conclusion, the administration of *P. pentosaceus* at a dose of $1x10^{10}$ CFUs lessens the severity of DSS-induced colitis and improves epithelial barrier function. It is safe and does not cause gastrointestinal disorders. However, the mechanisms through which *P. pentosaceus* improves the structure and function of the intestinal barrier need to be studied in depth. We also need to address whether this strain has any immunoregulatory effect on this model.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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