

Original Article

Stachyose modulates gut microbiota and alleviates dextran sulfate sodium-induced acute colitis in mice

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Abstract

Background/Aim: Ulcerative colitis (UC) has been implicated to imbalanced enteric flora and reduced microbial diversity. Stachyose is a kind of natural prebiotic which favorably modulate the composition of the gut microbiota. The present study aims to investigate the effects of stachyose on inflammatory levels and gut microbiota of acute colitis mice.

Materials and Methods: In this study, the mice were randomly divided into four groups: (1) control group; (2) stachyose group; (3) dextran sulfate sodium (DSS) group; (4) stachyose + DSS group. Hematoxylin and Eosin (H and E) staining was performed for the distal colon to examine the inflammation and tissue damage. The inflammatory cytokines including IL-6, IL-10, IL-17a, and TNF- α in serum were determined by ELISA assay. The differences in the gut microbiota were analyzed by 16S rDNA gene sequencing.

Results: Histological assay showed that the stachyose treatment significantly reduced the lesions of the colon in DSS-induced colitis. And the upregulated inflammatory cytokines induced by DSS were significantly inhibited by stachyose treatment. Additionally, the sequencing analysis showed that the stachyose changed the gut microbiota composition with a higher level of *Akkermansia*, as well as selectively increasing some probiotics, including *Lactobacillus*.

Conclusions: Our results suggested that stachyose increased beneficial microbiota and bacterial diversity to alleviate acute colitis in mice, which might be a new promising option to UC patients.

Keywords: Dextran sulfate sodium, gut microbiota, probiotics, stachyose, ulcerative colitis

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INTRODUCTION

Gut micro-ecosystem is a large and complex microbial community in the human body, including bacteria, fungi, viruses, parasites, and other eukaryotes.^[1] Under normal conditions, trillions of bacteria form a microbial barrier

to maintain the intestinal immune homeostasis, resist the invasion of pathogenic bacteria and assist in digestion and absorption of nutrients.^[2] In general, there is a dynamic balance between host and gut microbiota. It has been reported that microbiota imbalance is associated with

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various diseases, such as inflammatory bowel disease, diabetes, and depression. Several studies demonstrated that patients with ulcerative colitis (UC) who received fecal microbiota transplantation showed significant remission.^[3-5] It was suggested that the recovery and reconstruction of the gut microbiota might be an alternative therapeutic option for UC.

Stachyose is a tetrasaccharide present in the raffinose family of oligosaccharides with few side effects.^[6] It is a kind of natural prebiotic and its consumption is able to favorably modulate the composition of the gut microbiota in humans and animals.^[7,8] It is not decomposed by digestive enzymes and it will be changed in specific conditions when in direct contact with the intestinal tract.^[9] Stachyose can increase probiotic activity such as bifidobacteria and lactobacilli and regulate the balance of microecological flora in the human gastrointestinal tract. It can promote the formation of the dominant bacteria in the digestive tract and inhibit the production of spoilage bacteria such as *Clostridium*. Stachyose also promotes intestinal peristalsis and accelerates the excretion of pathogens and toxins.^[10]

Many studies showed that UC results from imbalanced enteric flora and reduced microbial diversity.^[11,12] Compared with healthy individuals, UC patients were observed to have decreased Firmicutes while bacteroidetes and proteobacteria increased.^[13,14] In addition, the performance of UC patients in different stages of intestinal flora is not the same. It is found that compared with patients in remission, active UC patients had a decreased number of lactobacilli, bifidobacteria, *Bacteroides* and increased number of *Escherichia coli*, *Enterococcus*, and *Clostridium* spp.^[15-17] Since the pathogenesis of UC involves an imbalance of bacteria, the strategies for regulating gut microbiota has gained increasing prominence.^[18]

Acute colitis mice model induced by dextran sulfate sodium (DSS) was similar to UC in symptoms and pathologic manifestations.^[19] In the present study, we explore the effects of stachyose on inflammatory levels and gut microbiota of acute colitis mice, to have a deeper understanding of microbiota and colitis.

MATERIALS AND METHODS

Animals

This study was ethically approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University on September 30, 2018 (SYSU-IACUC-2018-000189) and was conducted in conformity with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Twenty-eight male, 6–8 weeks old,

C57BL/6J mice weighing 18–20 g (purchased from Beijing Vital River Laboratory Animal Technology Company) were housed in specific pathogen-free conditions. The mice were maintained in a temperature-controlled (temperature, $25 \pm 2^\circ\text{C}$; relative humidity, $50 \pm 5\%$) facility with a 12-h light/dark cycle and free access to a standard mouse chow diet.

Experimental design

The mice were randomly divided into four groups: (1) a blank group which was neither given DSS (W/V) (36000–50000 kDa; MP Biomedicals, Solon, OH, USA) nor stachyose (YZ-4283, Extrasynthese, France); (2) stachyose group, which was only given stachyose (1.5 g/kg/day) for 28 days; (3) DSS group, which was only given 3% DSS drinking water for 7 days; (4) stachyose + DSS group, which was given stachyose (1.5 g/kg/day) for 28 days during the pretreatment (21 days prior) and period of acute colitis induced by 3% DSS in drinking water (*ad libitum* 7 days).

Histological studies

The distal colon segments from each animal were fixed in 4% paraformaldehyde (PFA), embedded in paraffin and cut into sections of 4 μm in thickness. The sections were then stained with hematoxylin and eosin (H and E). HE-stained sections were examined for inflammation and tissue damage.

Analysis of inflammation-related cytokines in serum by enzyme-linked immunosorbent assay (ELISA)

For the serum cytokine assay, blood from the eyeball was collected in a tube and centrifuged at $1100 \times g$, 4°C for 15 min. The serum was aspirated and assayed as described below. Concentrations of inflammatory-related cytokines IL-6, IL-10, IL-17a, and TNF- α in serum were measured by ELISA according to the manufacturer's instructions (Biolegend, San Diego, CA, USA). Briefly, biotinylated antibody reagent was added to 96-well plates, then supernatants of homogenized serum were added and the plates were incubated at 37°C in CO_2 for 2 h. After washing with phosphate-buffered saline (PBS), streptavidin-horseradish peroxidase (HRP) solution was added and the plate was incubated for 30 min at room temperature. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Analysis of microbiota composition by 16S rDNA gene sequencing

Total microbial DNA was extracted from the feces using the QIAamp DNA Stool Minikit (Qiagen Ltd, Strasse, Germany). The extracted DNA was used as the template to amplify the V3–V4 region of 16S rDNA genes by polymerase chain reaction (PCR), and the DNA libraries were

constructed by the Ion Plus Fragment Library Kit (Thermo Scientific, USA). The next-generation sequencing of 16S rDNA was performed by the Ion S5™ (Thermo Scientific, USA). Quality control was carried out through Fast-QC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Cluster reads into operational taxonomic units (OTUs) using Quantitative Insights into Microbial Ecology (QIIME, version 1.17) at the 97% sequence similarity. The α -diversity analysis was carried out to determine the species richness and species diversity, including the Chao1 index and Shannon index.

Statistical analysis

Continuous variables were described as mean \pm standard deviation, while categorical variables were presented as numbers and proportions. Difference between two groups was statistically examined through unpaired student t-test, and analysis of variance (ANOVA) was used for the statistical comparisons between multiple groups. *P* values were two-tailed, and *P* < 0.05 was considered statistically significant. All analyses were performed with Statistical Package for Social Sciences (SPSS; version 19, SPSS Inc., Chicago, IL, USA).

RESULTS

Effects of stachyose on histological damage in the colon tissue of mice with DSS-induced colitis

The colon length of four groups was presented in Figure 1. We found that the stachyose group had approximately 16.7% of the shortening rate of the colon while the DSS group had approximately 38.9%. After collecting colonic tissue samples, the severity of colitis was characterized by macroscopic examination of the colon and histological analysis of HE stained colonic sections. The inhibitory effects of the stachyose on the DSS-induced damage of the colon tissue are shown in Figure 2. HE staining

analysis indicated that the administration of DSS markedly increased the severity of the colitis compared with that in the normal mice [Figure 2a and b]. A typical lesion of the colon in the DSS administration group manifested multifocal areas, mucosal erosion, loss of epithelial and goblet cells, shortening and collapse of crypts, and submucosal edema. Stachyose administration reduced the lesions of the colon in DSS-induced colitis, as shown in Figure 2c and d. The protective and healing effects of stachyose on colon damage were more prominent.

Effect of stachyose on serum levels of IL-6, IL-10, IL-17a, and TNF- α

The IL-6 levels of control group, stachyose group, DSS group, and stachyose + DSS group were 30.58 ± 3.59 pg/mL, 17.75 ± 2.32 pg/mL, 672.45 ± 120.05 pg/mL, and 306.36 ± 59.39 pg/mL, respectively. The IL-6 level was significant between the DSS group and the stachyose + DSS group (*P* < 0.01). The IL-10 levels of control group, stachyose group, DSS group, and stachyose + DSS group were 13.68 ± 4.64 pg/mL, 7.61 ± 4.40 pg/mL, 184.67 ± 26.56 pg/mL, and 91.73 ± 3.62 pg/mL, respectively. The IL-10 level was significant between the DSS group and the stachyose + DSS group (*P* < 0.001). The IL-17a levels in control group, stachyose group, DSS group, and stachyose + DSS group were 2.15 ± 1.36 pg/mL, 1.78 ± 0.80 pg/mL, 19.27 ± 1.14 pg/mL, and 10.62 ± 0.79 pg/mL, respectively. The IL-17a level was significant between the DSS group and the stachyose + DSS group (*P* < 0.0001). The TNF- α levels in control group, stachyose group, DSS group, and stachyose + DSS group were 1.76 ± 0.33 pg/mL, 0.70 ± 0.43 pg/mL, 27.81 ± 1.41 pg/mL, and 13.78 ± 1.07 pg/mL, respectively. The TNF- α level was significant between the DSS group and the stachyose + DSS group (*P* < 0.0001). The results are presented in Figure 3.

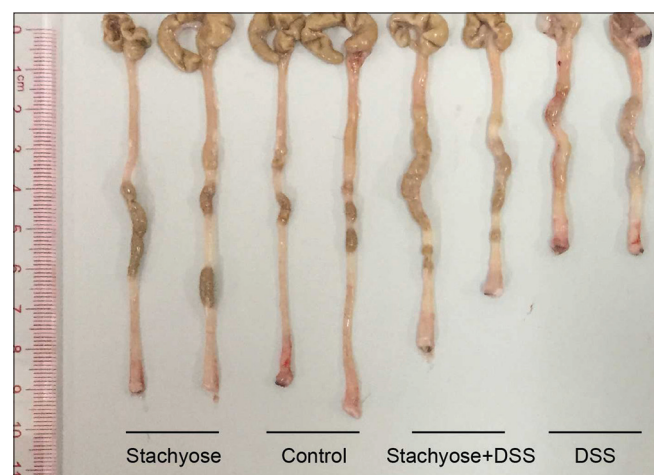


Figure 1: The colon length of four groups

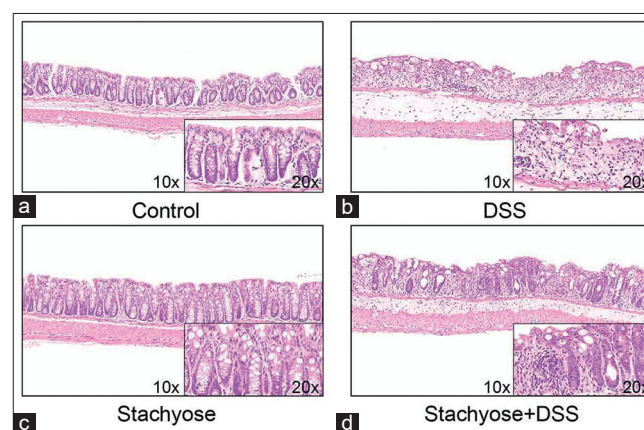


Figure 2: Effects of stachyose on histological damage in the colon tissue of mice with dextran sulfate sodium (DSS)-induced colitis. Histologic analysis of colon tissue for (a) control group, (b) DSS group, (c) Stachyose group and (d) stachyose + DSS group

Stachyose changed the gut microflora community composition in mice

The results of the taxonomic analysis are shown in Figure 4. At the genus level, *Escherichia-Shigella* was only detected in the DSS model and the DSS group was higher than the stachyose + DSS group (relative abundance: 0.175 vs 0.036). Stachyose group displayed higher *Akkermansia* than the control group (relative abundance: 0.102 vs 0.004). Besides, *Lactobacillus* was decreased in the control group compared with the stachyose group (relative abundance: 0.175 vs 0.355).

Alpha diversity in the DSS administration group was reduced. In addition, alpha diversity was more abundant

in the stachyose group than the control group, and alpha diversity was more abundant in the stachyose + DSS group than the DSS group. The chao1 index is shown in Figure 5.

DISCUSSION

The balance of gut microbiota plays a critical role in maintaining the mucosal barrier and immune function, which is involved with the production of antibacterial substances and the regulation of host immunity.^[11] Sellon *et al.* demonstrated that there was no intestinal inflammation in germ-free mice, and the mice underwent colitis after the recovery of gut microbiota.^[20] The incidence of UC

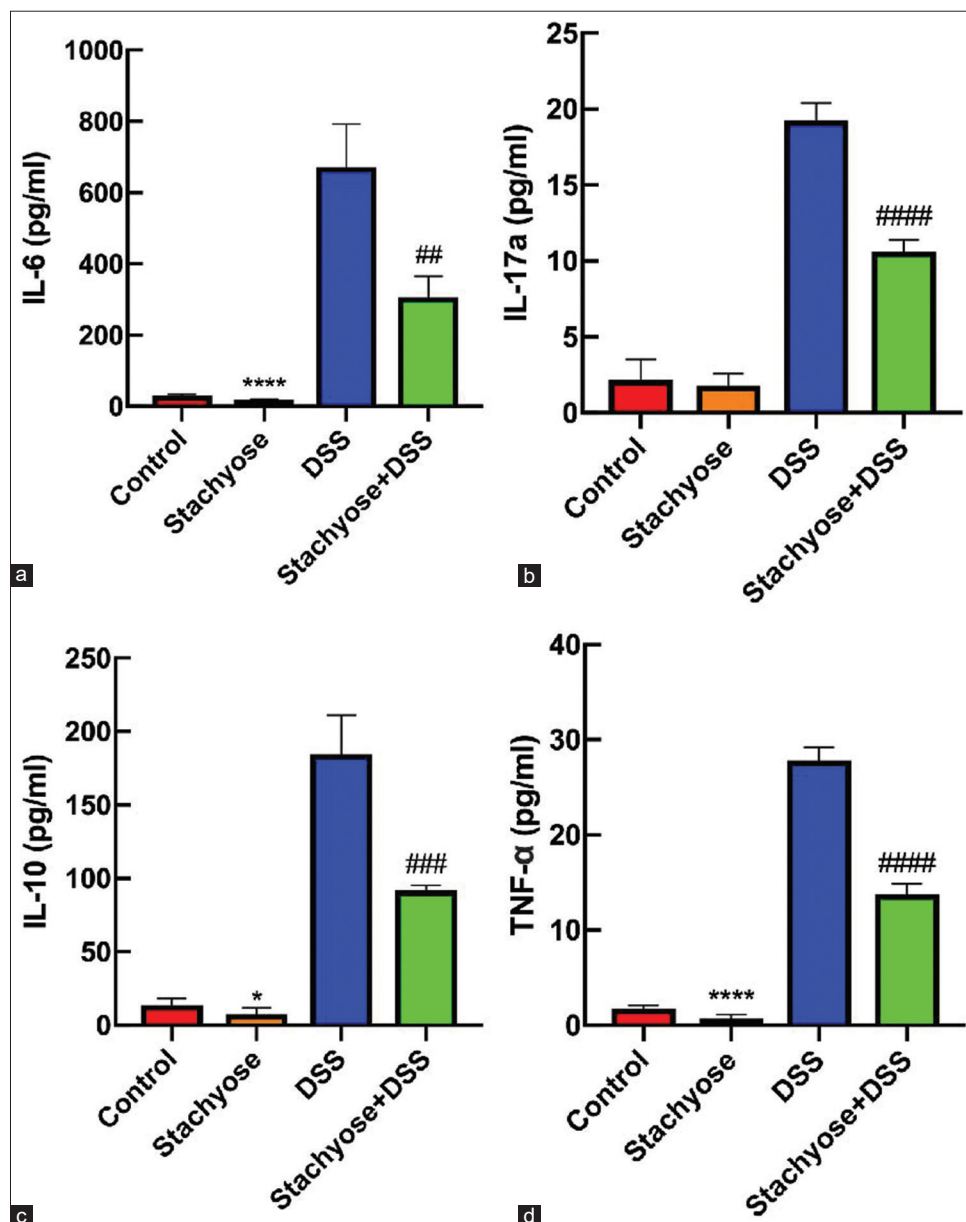


Figure 3: Effect of stachyose on serum levels of IL-6, IL-10, IL-17a, and TNF-α and #945; comparison of the serum IL-6 (a), IL-17a (b), IL-10 (c), and TNF-α and #945; (d) levels among the groups. The data are expressed as the means ± SD. **P* < 0.05; *****P* < 0.0001 vs control. ##*P* < 0.01; ###*P* < 0.001; ####*P* < 0.0001 vs DSS

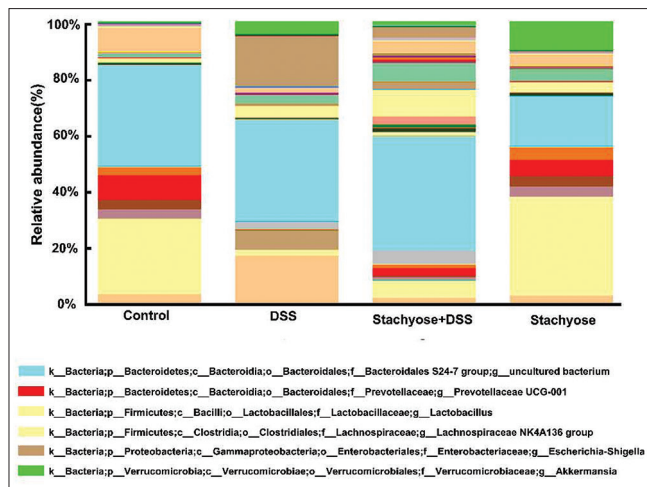


Figure 4: Composition of gut microbiota at the genus level in groups of mice

has gradually increased in the past 30 years, and studies have found that there exists a close relationship between the disease and intestinal flora.^[21] It was suggested that imbalanced intestinal microbiota was involved in the occurrence and development of UC. The mechanisms underlying the effects of imbalanced intestinal microbiota on UC may involve the stimulation of immune response and production of inflammatory cytokines. Manichanh *et al.* suggested that the levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* were significantly lower in patients with intestinal inflammation than those in healthy people.^[22] This study was conducted to investigate whether stachyose could alleviate the inflammatory levels and regulate the gut microbiota of acute colitis mice.

Prebiotics could promote the growth of probiotics such as *Lactobacillus* and *Bifidobacterium* to improve intestinal microecology and fight intestinal inflammation. There exists a large amount of research on probiotics while the impact of prebiotics on intestinal microbiota is

rarely studied. Qian *et al.* demonstrated that stachyose administration significantly alleviated DSS-induced colitis in mice,^[23] which was consistent with our result. Our study further investigated the role of stachyose in the intestinal microbiota of colitis mice through the 16s rDNA sequence.

Our results show that stachyose has protective and healing effects on colon damage. The addition of stachyose could reduce the level of serum IL-6, IL-10, IL-17a, and TNF- α . According to the results of the 16s rDNA sequence, we could find the increase and decrease of many genera. Among them, changes of *Lactobacillus* and *Akkermansia* were most significant. Our results indicate that *Lactobacillus* is obviously increased in the stachyose group compared with the control group, which is beneficial to the intestinal flora.

Stachyose group showed a higher level of *Akkermansia* than the control group, and the stachyose + DSS group had more abundant *Akkermansia* than DSS group, which suggested the promoting role of stachyose on the proliferation of *Akkermansia*. It was reported that *Akkermansia* participated in mucin degradation, production of short-chain fatty acids, and providing energy for the host.^[24] A recent study demonstrated that the abundance of *Akkermansia* showed a negative correlation with the level of IL-6 in serum,^[25] which was consistent with our result. Furthermore, studies showed that *Akkermansia* could improve inflammatory response in diabetic mice,^[26] protect intestinal epithelial cells, and maintain the mucosal barrier.^[27] Routy *et al.* demonstrated that the *Akkermansia* could ameliorate the antitumor effects of programmed death-1 (PD-1) blockade.^[28] It has been proved that *Akkermansia* could encode various secretory proteins such as sulfates, proteases, and glycohydrolase.^[29] In our study, *Escherichia-Shigella* was only detected in the DSS model and the DSS group was higher than the stachyose + DSS

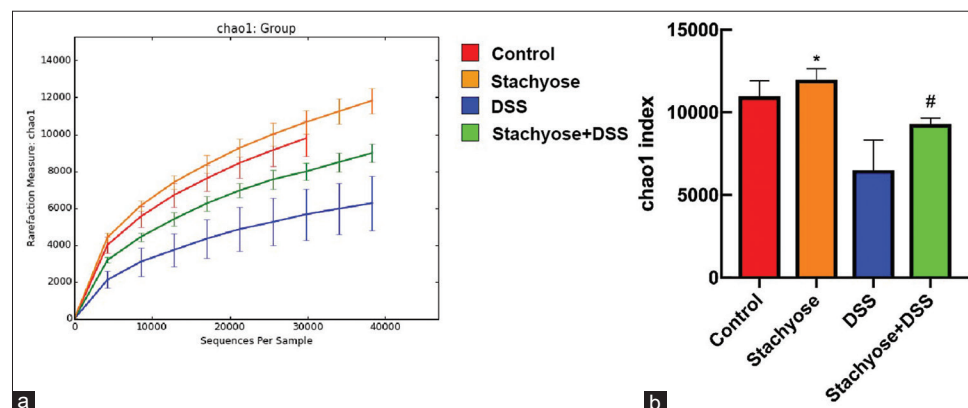


Figure 5: Stachyose modulated the microbial diversity in mice. (a) The chao1 curves, (b) Comparison of chao1 index among the groups. The data are expressed as the means \pm SD. * $P < 0.05$ vs Control. # $P < 0.05$ vs. DSS

group. Hence, we speculated that *Akkermansia* might decompose stachyose and facilitate its own proliferation and inhibit harmful bacteria. We also suggest that stachyose could increase the diversity of intestinal flora, which might be beneficial in reducing intestinal inflammation.

The effect of prebiotics in clinical patients has also been reported. For example, de Cossío *et al.* found that fructooligosaccharide could modulate microbiota in patients with metabolic syndrome,^[30] lactitol could increase the number of *Lactobacillus* and *Bifidobacterium*, and reduce the level of endotoxin in chronic hepatitis patients. However, the role of stachyose in patients with UC has not been previously reported. The analysis was carried out at the genus level because of inadequacy in species level. The effect of stachyose on mucous membrane flora is not observed, thus, further validation was still needed. Our results indicate that animal models could provide direction for clinical research to some extent, but could not completely simulate the physiologic and pathologic state of humans. In our study, the effects of stachyose on intestinal microbiota in acute colitis mice are preliminarily explored. The influence of different preparations on clinical patients and its mechanism still needs to be further studied to be better applied to patients.

In conclusion, this study found that stachyose increased beneficial microbiota and bacterial diversity to alleviate acute colitis in mice. It might be a new promising option for UC patients.

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Conflicts of interest

There are no conflicts of interest.

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