Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2023, **14**, 1627

Effects of *Nemacystus decipiens* polysaccharide on mice with antibiotic associated diarrhea and colon inflammation†

Haoyu Pan,[a,b,c] Xia Chen,[b,c] PeiPei Wang,[b,c] Junfeng Peng,[b,c,d] Judong Li[b,c,d] and Kan Ding *[a,b,c]

Antibiotic associated diarrhea (AAD) is a common side effect of antibiotic therapy in which gut microbiota plays an important role in the disease. However, the function of gut microbiota in this disease is still not entirely clear. Polysaccharides have shown strong activity in shaping gut microbiota. Whether the polysaccharide can intervene with the microbiota to improve ADD has not been determined. In this study, we extract crude polysaccharides from *Nemacystus decipiens* (*N. decipiens*), a traditional Chinese medicine (TCM), named NDH0. The crude polysaccharide NDH0 might significantly relieve the symptom of mice with AAD, including a reduction in body weight, shortening of cecum index and the infiltration of inflammatory cells into the colon. NDH0-treated mice exhibited more abundant gut microbial diversity; significantly increased the abundance of *Muribaculum*, *Lactobacillus*, and *Bifidobacterium* and decreased the abundance of *Enterobacter* and *Clostridioides* at genus level. NDH0 treatment down-regulated the level of pro-inflammatory cytokines, including IL-1β and IL-6 in colon tissue. NDH0 protected the integrity of colon tissues and partially inactivated the related inflammation pathway by maintaining occludin and SH2-containing Inositol 5′-Phosphatase (SHIP). NDH0 could alleviate symptoms of diarrhea by modulating gut microbiota composition, improving intestinal integrity and reducing inflammation. The underlying protective mechanism was to reduce the abundance of opportunistic pathogens and maintain SHIP protein expression. Collectively, our results demonstrated the role of NDH0 as a potential intestinal protective agent in gut dysbiosis.

Received 26th August 2021,
Accepted 27th December 2022

DOI: 10.1039/d1fo02813h

rsc.li/food-function

Introduction

Antibiotic-associated diarrhea (AAD) is caused by the abuse of broad-spectrum antibiotics, and it is suggested that a higher infection rate occurs as a consequence, which could cause severe diseases such as colitis, toxic megacolon, and even death.[1,2] Children are susceptible to diarrhea because of frequent antibiotic usage, and the incidence of AAD among children is about 20–35%.[1] Intestinal microbiota dysbiosis caused by antibiotic use contributes to the disease process.[3–5]

Microorganisms inhabiting the mammalian intestinal tract play an important role in maintaining gut homeostasis and host health.[6,7] Antibiotics significantly disrupt the composition and function of normal gut microbiota, leading to the development of obesity, allergic disease, and inflammatory bowel disease (IBD).[8–12] It has been widely found in studies that probiotic bacteria, *Bifidobacterium* and *Lactobacilli*, have various beneficial effects on human health, such as immune stimulation and mediation of metabolic functions.[13,14] Probiotics are often prescribed to prevent AAD as well.[15] Strains from numerous bacterial species have been examined in clinical studies for mitigating AAD, including members of *Bacillus*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus*, especially *Lactobacillus rhamnoses* strain GG.[16,17] Colonization by pathogens, such as *Enterococcus*, *Clostridium perfringens* and *Clostridium difficile*, might cause disease, including colitis and AAD.[4,13] Furthermore, altered microbiota composition precedes the onset of spontaneous ileitis in SHIP-deficient mice.[18] SHIP is a hematopoietic-specific lipid phosphatase that dephosphorylates PI3K-gene-rated PI (3,4,5)-trisphosphate, which is involved in regulating immune activation.[19–22]

[a]*Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, 1200 Cai Lun Road, Shanghai, 201203, China*
[b]*Glycochemistry and Glycobiology Lab, Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai, 201203, China*
[c]*University of Chinese Academy of Science, No. 19A Yuquan Road, Beijing, 100049, China*
[d]*Shanghai Changzheng Hospital, Naval Medical University, No.415 Fengyang Road, Shanghai, 200003, China*

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d1fo02813h

SHIP deficiency leads to reduced *Muribaculum intestinale* (*M. intestinale*) and intestinal inflammation in SHIP$^{-/-}$ mice.[18]

Traditional Chinese medicine (TCM) has been reported to be bioactive in anti-inflammation and alleviating various symptoms of diarrhea.[23,24] Polysaccharides are the main bioactive components of TCM and have anti-tumor and antioxidant effects, *etc*. *Nemacystus decipiens* polysaccharides are reported to have an antithrombotic effect by increasing the percentage of *t*-PA/PAI-1.[25] Our team extracted sulfated and acetylated fucoidan from seaweed in previous research. We showed that fucoidan from *Nemacystus decipiens* disrupted angiogenesis by down-regulating the expression of bone morphogenetic protein 4 (BMP4).[26] It has been reported that fucoidan extracts could ameliorate acute colitis and have anti-inflammation effects in mice.[27] However, it is not clear whether the polysaccharides from *Nemacystus decipiens* may have an impact on gut inflammation and diarrhea.

In this study, we examined the relevant growth performance (body weight and cecum index), gut microbiota composition, and intestinal protein expression in response to polysaccharide treatment for relieving AAD. We found that fucoidan has the ability to ameliorate symptoms of diarrhea and reduce the level of inflammation caused by AAD.

Materials and methods

Materials

Nemacystus decipiens was purchased from Jinying Marine Products Co., Ltd (Dalian province, China), originating from Okinawa (Japan) and identified by Doctor Peipei Wang. Clindamycin, ampicillin, ceftriaxone sodium and vancomycin were obtained from Dalian Meilun Biotechnology Co., Ltd (Dalian, China). A stool DNA kit was purchased from OMEGA Co. (Georgia, USA). Q Sepharose Fast Flow was obtained from GE Healthcare Life Science Co. (California, USA). A BCA protein assay kit was bought from Beijing Labgic Technology Co., Ltd (Beijing, China). Standard monosaccharides were purchased from Shanghai Aladdin Bio-Chem Technology Co. Ltd (Shanghai, China). Kits used in biology tests are shown in Table S1.† All other chemicals and reagents were obtained from China National Pharmaceutical Group Corp. (Shanghai, China).

Preparation of polysaccharides from *Nemacystus decipiens*

The polysaccharide sample used in this study was prepared and characterized as in our previous study.[26] Briefly, *N. decipiens* (5 kg) was sun dried before being percolated with 95% (v/v) ethanol. The residue (0.785 kg) was extracted with 80 °C water, followed by dialyzation (molecular weight cut off = 3500 Da) and precipitation by 80% ethanol. The precipitate was dried in a vacuum and washed with absolute ethanol and acetone twice. A Q Sepharose Fast Flow column was employed for polysaccharides while NDH0 was eluted with distilled water. High performance liquid chromatography (HPLC) equipped with tandem KS-804 and KS-802 columns (ID = 8 mm, length = 300 mm; Shodex Co., Tokyo, Japan) was employed for molecular weight detection. The monosaccharide composition of NDH0 was analysed by applying the PMP pre-column derivation method.[41] 2 M trifluoroacetic acid (TFA) was used to hydrolyse NDH0, followed by PMP derivation. The derivative was analyzed by HPLC equipped with an XDB-C18 column. Carbohydrate content was measured with a phenol–sulfuric acid colorimetric method using glucose as the standard, and protein content was measured using a BCA protein assay kit (Beijing Labgic Technology Co., Ltd, China). Sulfate groups were measured by the barium chloride gelatin method.[42]

Mice experiments

Animal experiments were approved and performed in accordance with the guidelines of Shanghai Institute of Material and Medica, Chinese Academy of Science (SIMM; Approval No. CGU11-117). Male mice of the C57BL/6NCrlBltw genetic lineage were purchased from Shanghai Ling Chang Biotechnology Co. Ltd (Shanghai, China) and kept under controlled light conditions (12 h light–dark cycle), with free access to food (the composition of food is shown in Table S2†) and water. After acclimatizing the animals, 24 4-week-old mice were randomly distributed into three groups (8 mice per group) as follows: control group (Control), antibiotic-associated diarrhea group (Model), NDH0 treatment group (NDH0). For antibiotic treatment, the model group and NDH0 group mice were treated with a 500 μg mL^{-1} mixture of antibiotics containing clindamycin, ampicillin, ceftriaxone sodium and vancomycin (ratio: 3 : 1 : 1 : 1) in free drinks for 1 week from day 0. The mice in the NDH0 group received 30 mg mL^{-1} NDH0 polysaccharide at 0.2 mL per mouse by gastric gavage once a day for two weeks from day 1. The mice of the control and model groups received an equivalent amount of physiological saline by gavage. Mice were weighed every two days from day 0. All samples were collected without fasting. The feces were collected on day 14 with sterile operation and immersed in liquid nitrogen, and then stored at −80 °C. Mice were anesthetized with isoflurane and then sacrificed. After sacrificing the mice, colon tissues were divided into two parts, stored in 4% paraformaldehyde for hematoxylin and eosin (H&E) staining and frozen in −80 °C to extract protein and mRNA. The cecum was rinsed with saline and weighed. The cecum index was calculated as follows:

$$\text{Cecum index} = \text{cecum weight (mg)}/\text{body weight (g)}.$$

Histological observation of colon

5 μm thick sections of each colon tissue were stained with H&E after flushing with water for 1 h, followed by dehydration with ethanol, and embedded in paraffin. Slides were visualized and photographed with an Axioscope 7 microscope with an AxioCamMR3 Digital Camera (Oberkochen, Germany).

Fecal DNA isolation

DNA extraction from collected feces was performed using an Omega Stool DNA Kit (Omega, USA), according to the manufacturer's instructions.

Colon tissue mRNA isolation and cDNA reverse transcription

Total RNA extraction from 100 mg of minced frozen tissues was performed using a Tiangen Kit (Beijing, China), according to the manufacturer's instructions. Then equal amounts of total RNA were used to synthesize cDNA.

Quantitative real-time reverse-transcription PCR

Quantitative real-time reverse-transcription PCR (q RT–PCR) was performed in triplicate using the ABI VIIA7 system with SYBR Green. Quantstudio Real-time PCR software was applied for data analysis. Relative quantification was done using the $2^{-\Delta\Delta CT}$ method. Expression was normalized against the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Mean expression levels of control group mice were set as 100%. The primers used are shown in Table S3.†

Microbial composition analysis

The V3–V4 region of the 16S rDNA gene was selected for PCR amplification using a forward primer (5′-ACTCCTACGGGAGGCAGCA-3′) and a reverse primer (5′-GGACTACHVGGGTWTCTAAT-3′). Gel electrophoresis and Vazyme VAHTSTM DNA Clean Beads were applied to purify the PCR products. The DNA library was constructed using a TruSeq Nano DNA LT Library Prep Kit. The DNA library was quantified with a TruSeq Nano DNA LT Library Prep Kit on the Promega QuantiFluor System. The optimized library was checked using an Agilent High Sensitivity DNA Kit before sequencing using the IlluminaMiSeq System. Quantitative insights from microbial ecology 2 (QIIME2) and R software packages (v3.2.0) were employed for sequence data analyses. The sequences with a similarity of >97% were defined as an operational taxonomic unit (OTU) using on-line QIIME software. Each representative sequence within each OTU was compared with the template sequence in the Greengenes database, and then the taxonomic information for each OTU was obtained.

Western blot

Total protein lysates were fractionated on a 10% sodium dodecyl sulfate–polyacrylamide gel and electro-blotted onto nitrocellulose filter membranes (Darmstadt, Germany). Membranes were blocked with 5% non-fat milk for 2 h at room temperature in TBST buffer (Tris 10 mM, NaCl 150 mM, pH 7.6, 0.1% Tween 20) and probed with primary antibodies overnight at 4 °C, followed by washing with TBST three times, each time for 10 minutes. Membranes were then incubated with horseradish peroxidase conjugated secondary antibody for 1 hour at room temperature. Primary antibodies were diluted into TBST at a ratio of 1 : 1000 and secondary antibodies were diluted at 1 : 5000. Protein bands were developed using enhanced chemiluminescence. The antibodies used are shown in Table S4.†

Statistical analysis

All data were expressed as means ± standard error of means (SEM). Statistical analyses were performed using SPSS Statistical 22 Software. Data sets involving more than two groups were assessed by one-way analysis of variance (ANOVA) followed by a non-parametric Kruskal–Wallis test with Dunn's multiple comparisons test. Values of $p < 0.05$ were considered statistically significant.

Results

Yield and physiochemical characterization of NDH0

NDH0 was obtained with a yield of 7.5% (w/w) and it consisted of carbohydrate with no protein detected. NDH0 was composed of fucose, glucuronic acid, mannose and xylose with a molar ratio of 83.4 : 10.2 : 3.1 : 3.2. The content of sulfate (as SO_4^{2-}) of NDH0 was measured as 11.4 ± 0.1% (m/m). Two polysaccharide fractions with average molecular weights of 329 kDa and 163 kDa were detected in NDH0 (Fig. S1†). The chemical composition of NDH0 was similar to NDH01, which was reported as a homogeneous fucoidan purified from NDH0.[26]

NDH0 may show robust diarrhea protective effects in AAD mice

To address whether NDH0 may inhibit diarrhea, we utilized a mixture of antibiotics to first set up an acute diarrhea mouse model. As body weight loss and increased cecum index are important indexes in an antibiotic associated diarrhea mouse model, these indexes are measured in Fig. 1. Mice were treated with a mixture of antibiotics in free drinks for one week to establish the diarrhea model, and the body weight gain of the model group of mice significantly decreased compared to the control group. However, after 7 days of gastric gavage with NDH0, the body weight gain of the NDH0 group was significantly increased compared to the model group. At the end of the experiment on day 14, mice treated with NDH0 had significantly higher body weight than the model group. The cecum index was measured to determine the severity of the diarrhea. Consistently, the cecum index of the model group was significantly increased compared with that of the control group. The NDH0 supplement significantly reduced the increase in the cecum index caused by the antibiotic mixture.

Histopathological analysis of colon tissues

To address whether NDH0 may inhibit inflammation and destruction of colon tissues caused by diarrhea, colon tissue H&E staining was measured. The histopathological changes of the colon in each group are shown in Fig. 2. AAD model mice exhibited epithelial disruption, increased edema and inflammatory cell infiltration into the submucosal layer, which were significantly ameliorated after NDH0 treatment. Furthermore, the colon villi of the AAD model mice were relatively short, and the intestinal mucosal epithelial cells were atrophied and desquamated compared with those of the control group. In contrast, compared with the model group, the colon villi of the NDH0 treatment group were smoother and closer to those of the control group.

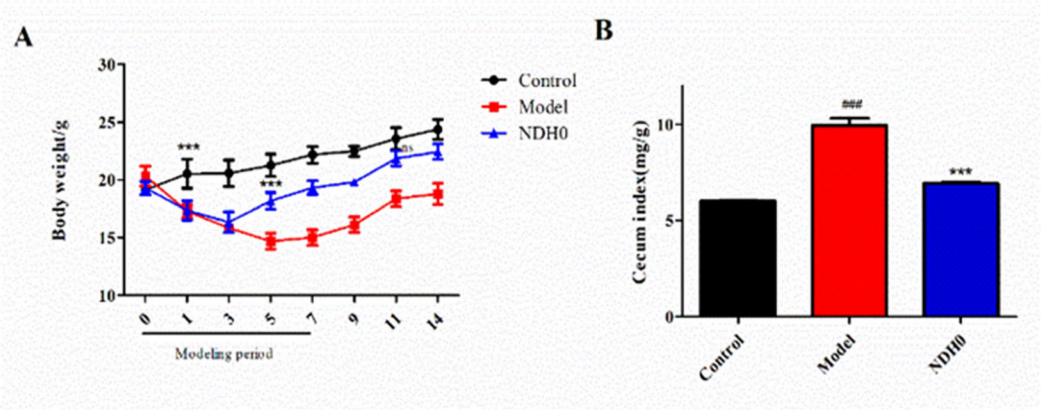

Fig. 1 Effect of NDH0 on body weight (A) and cecum index (B). Control (control group): mice were treated with saline from day 1 to day 14 by gavage; model (model group): mice were treated with a mixture of antibiotics (500 μg mL^{-1}) from day 0 to day 6 in free drinks and saline from day 1 to day 14 by gavage; NDH0 (NDH0 group): mice were treated with a mixture of antibiotics (500 μg mL^{-1}) from day 0 to day 6 and NDH0 polysaccharides (30 mg kg^{-1} d^{-1}) from day 1 to day 14 by gavage. Values are represented as means ± SEM (n = 8), ***p < 0.001 *vs.* model, ###p < 0.001 *vs.* control. Raw data is shown in Table S5.†

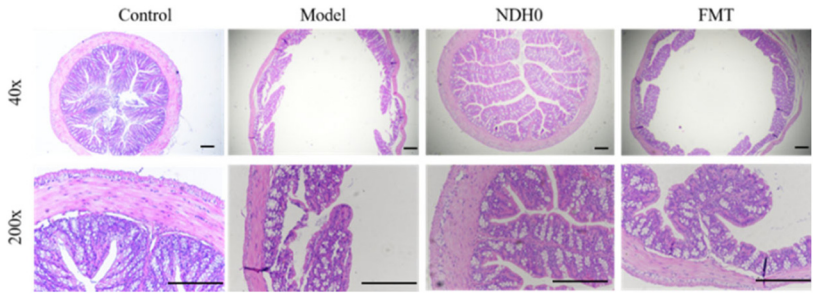

Fig. 2 Histopathological examination of the colon tissues. Control: control group; Model: model group; NDH0: NDH0 group.

NDH0 alleviates antibiotic-induced gut dysbiosis

To determine whether NDH0 may shape gut microbiota after antibiotics caused microbiota dysbiosis, we performed 16S rDNA sequencing to detect the bacterial changes. A profound alteration of the gut microbiota occurred in the host intestinal tract. In Fig. 3, we found antibiotics disturbed the gut microbiota significantly in the model group. Chao1 and Observed_species indices presented the richness of the microbiota and Shannon and Simpson indices presented the diversity of gut microbiota. In Fig. 3A, the results showed that the richness and diversity of the gut microbiota in mice treated with the antibiotic mixture were significantly reduced compared to the control group. These results also revealed that the richness and diversity of the gut microbiota were restored in mice from the NDH0 treatment group. Principal co-ordinates analysis (PCoA) was used to evaluate the clustering of the gut microbiota in each group (Fig. 3B). A plot of the PCoA scores showed that the model group had a significant shift along PC01 compared with the Control and NDH0 groups. Control, NDH0 and model groups showed a gradual slight structural shift along PC2. The NDH0 group was closer to the control group than the model group. The relative abundance of the gut microbiota classification units is shown in Fig. 3C, D and Table 1. At the phylum level, the main composition content was *Bacteroidetes*, *Firmicutes* and *Proteobacteria* in all groups. Mice treated with antibiotics showed a significant increase in *Proteobacteria* and a decrease in *Bacteroidetes* and *Firmicutes*. NDH0 treatment restored the dysbiosis. Although the abundance of *Proteobacteria* was higher than that of the control group, it was still significantly decreased compared to the model group, and the abundance of *Bacteroidetes* returned to normal levels in the NDH0 group. At the genus level (Fig. 3D and E), the abundance of *Muribaculum*, *Lactobacillus*, *Ruminococcs* and *Bifidobacterium* was significantly decreased and *Enterobacter*, *Clostridioides*, *Blautia*, and *Enterococcus* were significantly increased in the model group compared with the control group. Compared to the model group, the abundance of *Enterobacter* and *Clostridioide* were significantly decreased in the NDH0 group. NDH0 treatment restored the altered gut microbiota composition, and the gut microbiota composition of NDH0 group was more similar to that of the control group.

NDH0 attenuates AAD-associated inflammation levels in diarrhea mice

Emerging explorations of the interaction between polysaccharides and gut microbiota have provided us with new insights

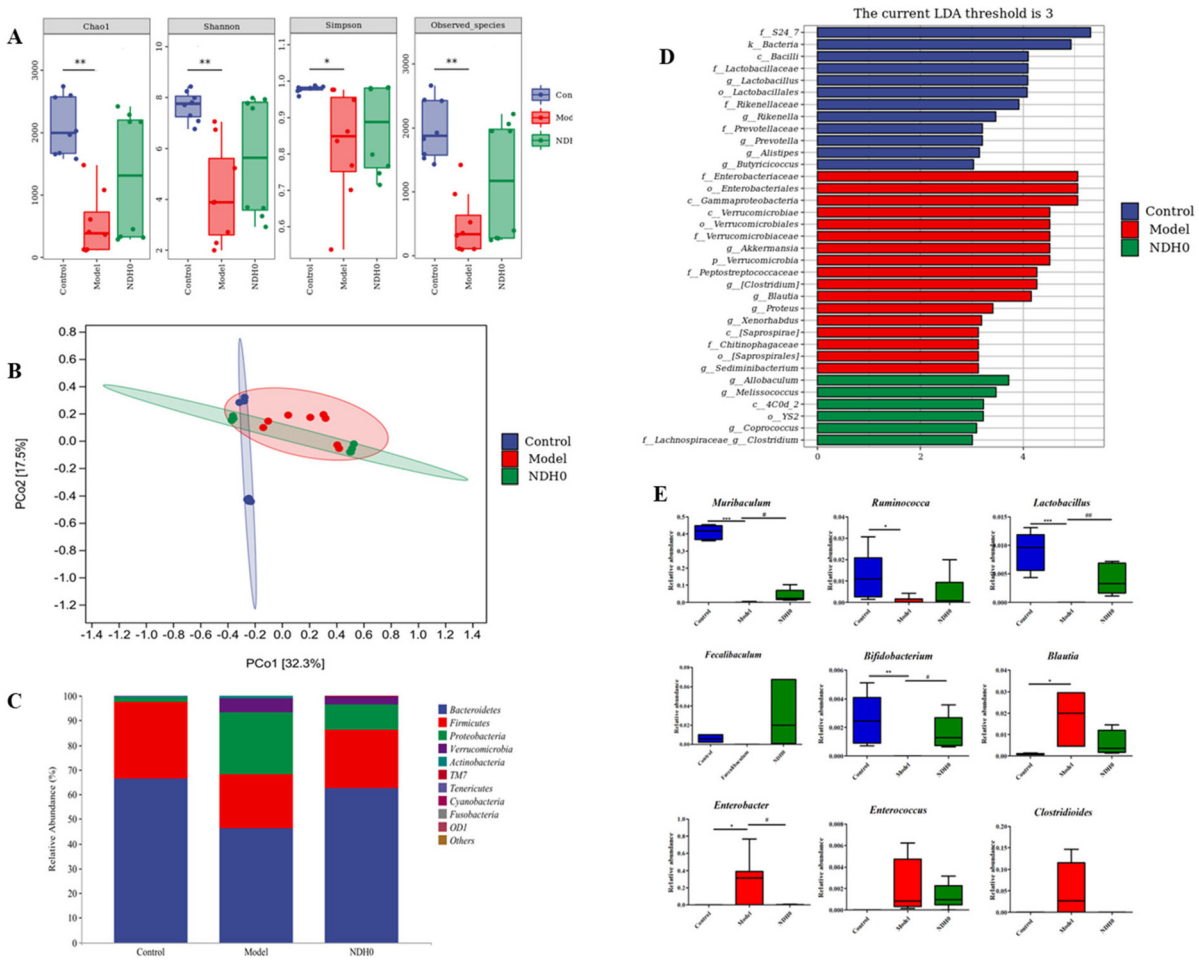

Fig. 3 The effect of NDH0 on mice gut microbiota. α diversity and composition of gut microbiota. (A) Alpha diversity boxplot (Chao1, Shannon, Simpson, Observed_species). (B) Principal co-ordinates analysis (PCoA). (C) Composition of gut microbiota at phylum level. (D) LDA score computed from features differentially abundant between three groups. (E) Main different bacteria at genus level. Raw data of different bacteria at genus levels is shown in Table S7.† Control: control group; model: model group; NDH0: NDH0 group. ***$p < 0.001$ *vs.* control, #$p < 0.05$ *vs.* model, $n = 5$.

Table 1 Relative abundance of gut microbiota at the phylum level in each group. Control: control group; Model: model group; NDH0: NDH0 polysaccharide group. Data are expressed as means ± SEM ($n = 5$)*, *$p < 0.05$, **$p < 0.01$ *vs.* Model. Raw data is shown in Table S6†

	Group		
Relative abundance	Control	Model	NDH0
Bacteroidetes	74.84 ± 4.45%*	24.47 ± 15.00%	54.77 ± 11.97%
Proteobacteria	2.52 ± 0.36%**	70.77 ± 15.29%	31.35 ± 12.52%
Firmicutes	21.97 ± 4.80%*	4.72 ± 2.57%	8.32 ± 1.78%

into its mechanism. It has been reported that SHIP deficiency leads to reduced *Muribaculum intestinale* before intestine inflammation occurs.[18] In this part, we wanted to figure out whether antibiotics or NDH0 could affect the SHIP and occludin in colon tissues. The expression of SHIP was analyzed by two different primers. The results showed that SHIP was significantly decreased in the model group compared to the control group; however, it increased in the NDH0 group compared to the model group (Fig. 4A and B). We also detected the SHIP and downstream immune-related proteins by western blotting to figure out the mechanism of NDH0 inhibiting inflammation. As the western blotting results show, the protein level of SHIP was decreased, and PI3K, phosphorylated PI3K, NF-κB, and phosphorylated NF-κB were increased in the model group compared to the control group. However, NDH0 treatment might significantly decrease the phosphorylated PI3K and phosphorylated NF-κB level. Nevertheless, the increase in SHIP level was not significant after the treatment with NDH0. The changes in these molecules in western blot correspond to the results of the histopathological examination.

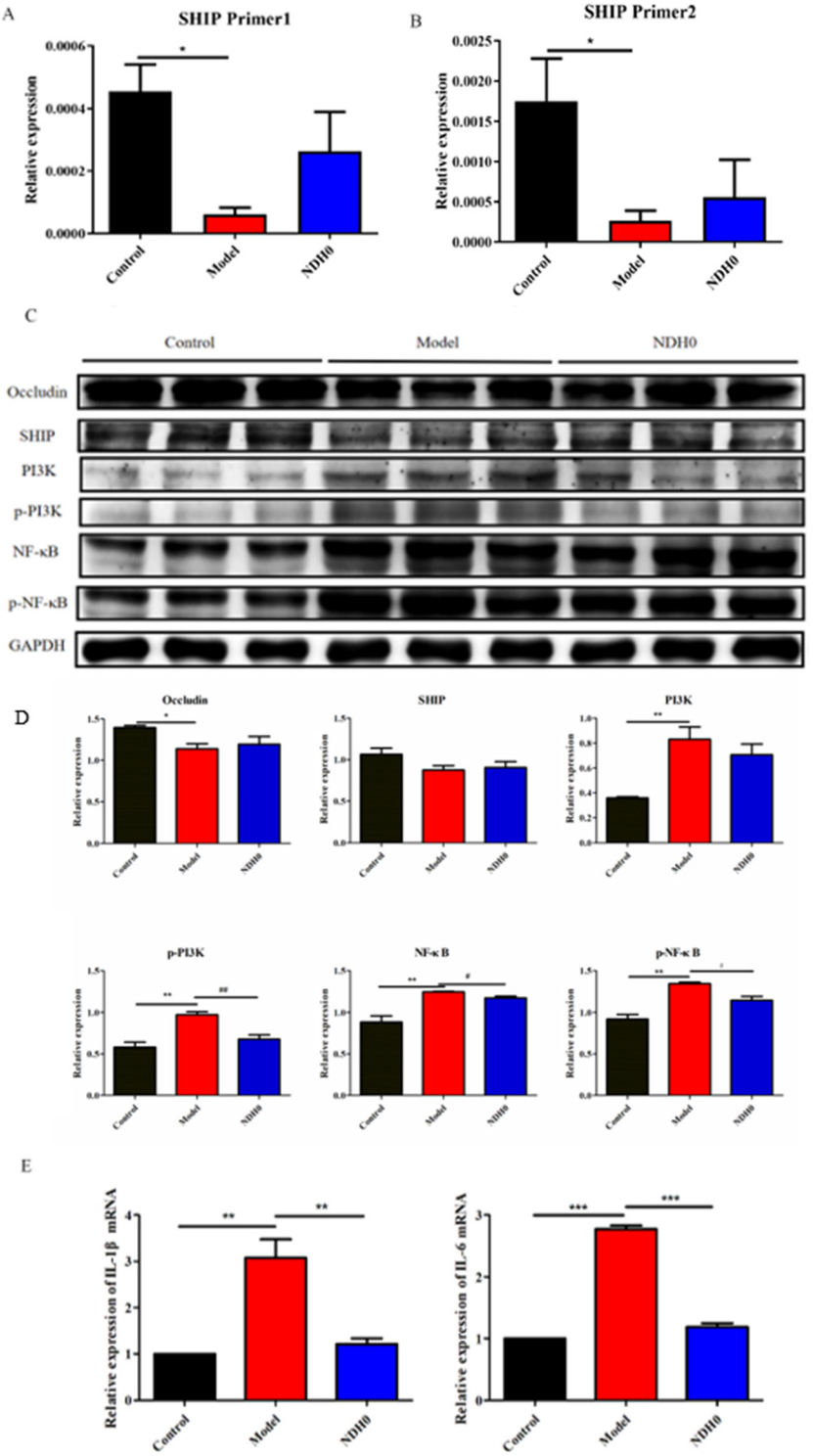

Fig. 4 NDH0 reduced the inflammation by protecting gut permeability and immunomodulatory protein in colon tissues. (A and B) SHIP mRNA relative expression analyzed by RT-qPCR using different primers. (C) The expression of occludin, SHIP, PI3K, p-PI3K, NF-κB, p-NF-κB detected by western blot (WB) with GADPH as internal control. (D) The relative expression levels of occludin, SHIP, PI3K, p-PI3K, NF-κB, p-NF-κB deduced from the WB results. (E) IL-1 and IL-6 mRNA relative expression analyzed by RT-qPCR. Error bars represent mean ± SEM (n = 3). Statistical analysis of the quantitative multiple group comparisons was performed using the one-way analysis of variance (ANOVA) followed by Tukey's test, *p < 0.05, **p < 0.01, ***p < 0.001 *vs.* model. Raw images of data from western blot are shown in Fig. S2.†

Excessive mucosal permeability may be a key cause of AAD. Thus, we detected the occludin level in colon tissues, which is a tight junction protein affecting epithelial permeability status. As the results show, occludin was decreased in mice treated with antibiotics, suggesting abnormal mucosal permeability, while NDH0 treatment could restore the level of occlusion (Fig. 4C). Furthermore, in each group of animals, we assessed the transcriptional expression level of two inflammatory cytokines, IL-1 and IL-6, which could represent the inflammation level in the colon. The results showed that IL-1 and IL-6 were significantly increased in the model group compared to the control group (Fig. 4E). As expected, the IL-1 and IL-6 mRNA levels were decreased after NDH0 treatment.

Discussion

There is a high prevalence rate of antibiotic associated diarrhea when antibiotic therapy is performed. The mechanisms by which antibiotics lead to AAD are *Clostridium difficile* infection, dysbiosis of the composition and function of gut microbiota, and the toxic effects of antibiotics on intestinal mucosa or pharmacologic effects on motility.[28]

In recent years, people have mainly focused on *Clostridium difficile* polysaccharides from the red seaweed *Gelidium pacificum* Okamura infection or altered gut microbiota composition with next-generation sequencing. Recently, probiotics showing the ability to alter gut microbiota, supporting intestinal barrier function, and influencing the immune system in AAD, are frequently prescribed with antibiotic use in clinical practice.[15] Chinese traditional medicine also attracts researchers' attention as a potential therapy in AAD. It has been reported that *Schisandra chinensis* polysaccharides have beneficial effects on rats with AAD.[24] Furthermore, sulfated polysaccharides from the red seaweed *Gelidium pacificum* Okamura showed beneficial effects on mice with AAD.[29] However, the mechanisms of gut microbiota alleviating diarrhea symptoms and level of inflammation in the colon are still not clear. In this study, NDH0, a sulfated polysaccharide in which the monosaccharide component is fucose, shows a good effect on mice diarrhea and anti-inflammation activity. *Clostridium difficile* infection is considered a key factor in diarrhea.[1,30] Gut microbiota, especially *Lactobacillus* and *Bifidobacterium* strains, have the ability to suppress the growth of *Clostridium difficile.*[31] A microbiota is defined as the assemblage of microorganisms existing in a particular environment, including the microorganisms and the environmental conditions of that habitat.[43] The gut microbiota performs a defensive function by competing for attachment sites and nutrients with opportunistic pathogens, such as *Clostridioides*, thereby preventing invasion or overgrowth by these organisms.[44] Antibiotics could disrupt the balance that normally exists between the various species of gut microbiota.[45] For instance, antibiotics can lead to the overgrowth of pathobionts, such as toxigenic *Clostridium difficile*, by causing a decrease in species diversity.[46] In this study, the relative abundance of *Clostridioides* is increased in the model group. However, the relative abundance of *Lactobacillus* and *Bifidobacterium* also increased in the NDH0 group. Although the results indicate that beneficial bacteria may not reduce the abundance of *Clostridioides*, they could improve the status of diarrhea. Other opportunistic pathogens, such as *Clostridium perfringens*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, and *Candida* species, have also been associated with AAD.[4] In this study, we found that the relative abundance of *Enterobacter*, *Blautia*, and *Enterococcus* increased in the model group. Meanwhile, the abundance of *Enterobacter* is negatively associated with levels of *Lactobacillus* and *Bifidobacterium*, suggesting that a high level of *Enterobacter* may be the key factor in diarrhea and *Lactobacillus* and *Bifidobacterium* could suppress its growth.

Integrity of the intestinal epithelial barrier plays a crucial role in intestinal diseases.[32,33] Excessive mucosal permeability is observed after intestinal injury induced by antibiotics and the occluding level is decreased after treatment with antibiotics.[34] It has been reported that polysaccharides extracted from Chinese traditional medicine could improve the integrity of the intestinal epithelium.[35,36] In this study, we observed that the tight junction protein occludin decreased in the model group, whereas NDH0 maintained the expression of occludin. These findings suggest that NDH0 might relieve the symptoms of AAD by modulating the composition of gut microbiota and maintaining the integrity of the intestinal epithelial barrier.

Inflammation is an important feature of AAD because antibiotics could affect immune homeostasis. Besides, antibiotics may induce inflammation and heighten susceptibility to some infections.[37] It has been reported that bacteria have the ability to counter the antibiotic activation of inflammation.[38,39] NDH0 may play an important role in regulating gut microbiota to prevent the side-effects of antibiotics. We find that the abundance of *Muribaculum* decreased in the model group, while it increased in the NDH0 group. Interestingly the abundance of *Muribaculum intestinale* is reduced in SHIP-deficient mice with spontaneous ileitis,[18] while SHIP is a signaling protein component of many growth factor receptor signaling pathways.[22] SHIP has been referred to as a "gatekeeper" because it negatively regulates multiple downstream receptors in mast cell activation.[21] In this study, we found that antibiotics suppressed the expression of SHIP in colon tissues, whereas NDH0 augmented the expression of SHIP. Furthermore, the downstream of the inflammatory pathway is activated in the model group. However, NDH0 might reverse the abnormal activation. Cytokines play an important role in the immune system, reflecting the inflammatory status of the host. A negative correlation has been reported between the levels of IL-1β, IL-6 and fiber intake.[40,42] In our study, the level of IL-1 and IL-6 increase in the model group was decreased after NDH0 treatment. These results reveal that by maintaining the integrity of the intestinal epithelium NDH0 may contribute to alleviating the level of colon inflammation.

Conclusions

Our results show that *Nemacystus decipiens* polysaccharides, NDH0, might alleviate diarrhea symptoms through modulating the composition of gut microbiota, improving intestinal integrity and reducing inflammation.

Author contributions

Haoyu Pan: writing-original draft, visualization, investigation, data curation. Xia Chen: resources and revision. Peipei Wang: resources. Junfeng Peng: investigation. Judong Li: investigation. Kan Ding: conceptualization, supervision, writing – review & editing.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by National Natural Science Foundation of China (81903762) and Key New Drug Creation and Manufacturing Program (2019ZX09735001), Ministry of Science and Technology, the People's Republic of China.

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