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Seleno-chitooligosaccharide-induced modulation of intestinal barrier function: Role of inflammatory cytokines, tight junction proteins, and gut microbiota in mice

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Abstract
This study aimed to explore the function of Seleno-chitooligosaccharide (SOA) on the intestinal barrier through regulation of inflammatory cytokines, tight junction protein, and gut microbiota in mice. The results of ELISA assay demonstrated that SOA significantly increased the levels of IL-2, IL-10, and IFN-γ in serum and ileum. Meanwhile, SOA increased the levels of IL-4 in the ileum ($p < 0.05$). In addition, Diamine Oxidase (DAO) concentration was decreased in ileum by SOA treatments ($p < 0.05$). The administration of SOA significantly upregulated the expression of ZO-1 and Occludin in the ileum ($p < 0.05$). By 16S rDNA sequencing, reduced ratio of *Bacillota/Bacteroidota* was observed in SOA treated mice. Within the phylum of *Bacteroidota*, SOA increased the relative abundance of *Deferribacterota*, uncultured *Bacteroidales* bacterium, and *Bacteroides*. Within the phylum of *Bacillota*, increased relative abundance of *Erysipelatoclostridium* and *Lachnoclostridium*, and reduced relative abundance of *Ruminococcaceae* UCG-010 were observed with SOA supplement. In summary, SOA has the potential to modulate the function of intestinal barrier function and prevent intestinal diseases.

Keywords: Gut microbiota; Inflammatory; Intestinal barrier; Seleno-chitooligosaccharide (SOA)

Highlights:
- Seleno-chitooligosaccharide (SOA) regulates immune cytokines to activate immune response.
- The expression of tight junction proteins (ZO-1 and Occludin) was upregulated by SOA treatment.
- Reduced ratio of *Firmicutes/Bacteroidetes* was observed in the situation of SOA treated mice.
- SOA increased the relative abundance of *Deferribacteres*, uncultured *Bacteroidales* bacterium, *Bacteroides, Erysipelatoclostridium* and *Lachnoclostridium*, and reduced the relative abundance of *Ruminococcaceae*.

Introduction

The intestinal barrier, a critical interface between the external and internal human environments, consists of protective layers including mechanical, chemical, biological, and immunological components (Schoultz and Keita, 2020). It primarily ensures containment of undesirable luminal contents within the intestine while preserving the ability to absorb nutrients. As reported by Chelakkot et al. (2018) and Iliev et al. (2025), barrier dysfunction enhances the absorption of antigens, toxins, and pro-inflammatory molecules, contributing to diseases like Crohn's disease, food allergies, viral and bacterial gastroenteritis, ulcerative colitis, and multiple organ dysfunction syndromes (Clarke and Chintanaboina, 2019; Sartor, 2006). Therapies targeting barrier dysfunction are under development, despite associated side effects, toxicity, and costs (Kong et al., 2024; Qiu et al., 2024; Tao et al., 2025).

Tight junction proteins are essential for maintaining mucosal selectivity, with dysregulation leading to increased permeability and systemic entry of harmful substances (Chelakkot et al., 2018). The gut microbiota, a biological barrier, contributes to health homeostasis, with dysbiosis linked to various diseases, including Parkinson's and colorectal cancer, as indicated by changes in the *Bacillota/Bacteroidota* ratio (Chen et al., 2021). Thus, the interplay between the intestinal barrier, tight junction proteins, and gut microbiota highlights the need for comprehensive strategies to preserve gut health and prevent disorders.

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Selenium, an essential trace element for human health, is intrinsically incorporated into the structure of selenoproteins. Selenoproteins are a unique group of proteins in which selenocysteine serves as the 21st amino acid (Roman et al., 2014). These proteins coordinate key physiological processes, including a vital role in antioxidant defense, oxidation-reduction (redox) homeostasis, and the immune system (Rayman, 2012). Clinical evidence indicates that selenium deficiency can exacerbate intestinal barrier dysfunction and is closely linked to inflammatory bowel disease (IBD) (Kudva et al., 2015). Appropriate intake of selenium has been shown to have therapeutic benefits for a variety of diseases. For instance, studies have suggested that selenium may play a role in the management of gastrointestinal carcinoid, prostate cancer, cardiovascular disease, diabetes, and diseases of the reproductive system (Méplan and Hughes, 2020). In recent years, more and more researchers have been working on selenium supplementation, and revealed that organic selenium has lower toxicity and higher biological activity than inorganic selenium (Ferrari et al., 2023; Kieliszek, 2019). Selenium is of pivotal importance in sustaining human health and in the prevention and treatment of various diseases. Among selenium-containing compounds, a characteristic organic selenium compound – selenopolysaccharide – has garnered substantial attention on account of its diverse and advantageous physiological functions, including antiviral, antitumor, and antioxidant properties (Duan et al., 2022).

Chitooligosaccharide, the hydrolyzed derivative of chitosan, also known as amino oligosaccharide, has significant biological activity at the level of cytology and molecular biology. Studies have documented its multifaceted pharmacological properties, including anti-obesity and lipid-lowering, antidiabetic, antihypertensive, immunomodulatory, and anticancer effects (Zhai et al., 2021). Investigations have revealed that chitooligosaccharides may modulate the expression and functionality of tight junction proteins, thereby contributing to the enhancement of intestinal barrier function (Li et al., 2024). Chitooligosaccharides and their preparations mitigated intestinal barrier damage and enhanced tight junction protein expression by targeting the regulation of microbiota related to intestinal barrier and mucosal immunity, thereby slowing down kidney damage (Zhang et al., 2025). Chitooligosaccharides interact with the intestinal microbiota to promote its growth and regulate the microbial community, which have a positive impact on the maintenance of intestinal barrier function.

Our previous study has established the view that low molecular weight seleno-aminopolysaccharide has a protective effect on the intestinal barrier (Wen et al., 2018). Studying the effects of seleno-chitooligosaccharide (SOA) on intestinal barrier function, especially with a focus on the role of inflammatory cytokines, tight junction proteins, and intestinal flora in mice, has important implications for understanding its potential therapeutic applications in gut-related diseases. Therefore, this work provides new insights into the role of SOA in improving the function of the intestinal barrier in an *in vivo* mouse model by characterizing changes in serum cytokines, ileum cytokines, and gut microbiota.

Materials and methods

Materials and reagents

SOA was self-synthesized in the laboratory of Zhejiang Ocean University. Briefly, chitooligosaccharide (3 kDa, 1 g) was dissolved in 30 ml of 2% (w/v) acetic acid solution and sodium selenite (1.2 g) was dissolved in 10 ml of 2% (w/v) acetic acid solution. The two liquids were stirred to clear, then mixed together, let to react at 70 °C for 4 h, dialyzed for 24 h, and freeze-dried for 24–48 h. The total selenium content in SOA was about 3.175 mg/g.

Animal's care and experimental study design

All animal experiments were conducted on male ICR mice (initial age 6 weeks) obtained from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China). All mice were kept in individual ventilated cages, in the Animal Laboratory Center of Zhejiang Ocean University in China (22 ± 2 °C, 40–70%, following a 12 hours light/dark cycle), with food and water provided *ad libitum*. All the animal procedures were approved by the Committee on the Ethics of Animal Experiments of Zhejiang Ocean University in China [Approval No. SYXK-(ZHE) 2019-0031, Permission No. 2023031].

After acclimatization, mice were randomly divided into two groups (n = 8 per group): the control group (CON) and SOA treated group (SOA). The mice in the SOA group were orally administered with SOA (200 mg/kg; the dose of SOA was determined according to the actual body weight) for 21 days, while the control animals received daily gavage with distilled water. All mice were weighed daily to track weight gain/loss.

After 21 days of treatments, all mice were anaesthetized with pentobarbital sodium and the blood samples, ileum, cecum content were collected and stored at –80 °C for further analysis.

Measurements of IL-2, IL-4, IL-10, IFN-γ, and Diamine Oxidase (DAO)

After 12 h fasting and anesthesia, the blood was obtained, centrifuged at 3,000 × g for 10 min and the serum was collected. 100 mg of ileum tissue was homogenized in 1 ml normal saline solution and centrifuged at 1,700 × g for 10 min at 4 °C. The concentrations of IL-2, IL-4, IL-10, IFN-γ, and DAO in serum and ileum were assessed by ELISA assay, according to the ELISA kit instructions respectively (Boshide Bioengineering Limited Company, Wuhan, China).

Quantitative Real-Time PCR (qRT-PCR)

The changes in mRNA expression of IL-2, IL-4, IL-10, INF-γ, ZO-1, and Occludin relative to β-actin were assessed in ileum tissue. Total RNA was isolated from liquid nitrogen frozen ileum by using ice-cold TRIZOL reagent (Thermo Fisher Scientific, NY, USA) following the supplier's protocol. RNA quality and purity were determined using NanoPhotometer (Implen, Germany). PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, Japan) was used to synthesize the first strand of cDNA following the manufacturer's instructions. The sequences of the primers and conditions are listed in Table 1. The reactions were carried out by Applied Biosystems ViiA™ 7 Real-time PCR system (Thermo Fisher Scientific, New York, USA). For each reaction, 2 μl cDNA were diluted to an appropriate concentration by adding directly to SYBR Green Mix (Takara, Japan) following the manufacturer's instructions. The qRT-PCR reactions were performed as follows: 40 cycles of denaturization at 95 °C for 15 s, annealing at corresponding melting temperature for 60 s, and extending at 72 °C for 20 s. Relative gene expression was calculated by using $2^{-\Delta\Delta Ct}$ method.

16S rDNA sequencing of gut microbiota

The total DNA from cecum content was extracted using a FastDNA™ SPIN kit (MP Biomedicals, CA, USA). DNA con-

Table 1. Quantitative Real-Time PCR Primers and conditions

Gene	Gene accession number	Primer sequence 5'-3'	PCR product size (bp)	Tm
IL-2	NM_008366	F: TCAGCAACTGTGGTGGACTT R: GCCTTATGTGTTGTAAGCAGGA	106	64
IL-4	NM_021283	F: GTTCTTCGTTGCTGTGAGGAC R: TGTACCAGGAGCCATATCCAC	135	66
IL-10	NM_010548	F: CAGTCGGCCAGAGCCACAT R: CTTGGCAACCCAAGTAACCCTT	144	64
IFN-γ	NM_008337	F: TAACTCAAGTGGCATAGATGTGGAAG R: GACGCTTATGTTGTTGCTGATGG	169	64
ZO-1	XM_006540786.3	F: CCCGAAACTGATGCTGTGGATA R: CCCTTGGAATGTATGTGGAGAGAA	146	60
Occludin	XM_011244634.2	F: GCCCAGGCTTCTGGATCTATGT R: GGGGATCAACCACACAGTAGTGA	124	61
β-actin	NM_007393	F: AGTGTGACGTTGACATCCGT R: GCAGCTCAGTAACAGTCCGC	298	60

Note: F, forward; R, reverse

centration and purity were measured by NanoPhotometer and DNA integrity was measured by agarose gel electrophoresis. The V3-V4 region was amplified using barcoded primers (338F: 5'-ACTCCTACGGGAGGCAGCAG-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). PCR amplification was performed as follows: pre-denature, 95 °C for 30 s, 40 cycles of 95 °C for 5 s, 54 °C for 34 s and 72 °C for 45 s, and subsequent 72 °C for 10 min. The amplicons were then purified using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, China) and quantified by Quantus™ Fluorometer. Illumina MiSeq sequencing platform was used for high-throughput sequencing of PCR products from samples. Raw reads were analyzed using the software package of QIIME2 (V1.8.0). The remaining quality-filtered reads were gathered into Operational Taxonomic Units (OTUs) with a 97% similarity cut-off by USEARCH v7.0. The α diversity analysis was performed by calculating the Good's coverage, Chao, ACE, Shannon and Simpson indices in the sample using the QIIME (V1.7.0) software package, and the results were visualized using the R (V3.4) software. Good's coverage was used to characterize the sequencing depth of the sample, Chao and ACE indices were used to identify community richness. The Shannon and Simpson index were used to identify community diversity. The composition of OTUs in intestinal microbiota of samples from different groups was compared using R software package (V3.4).

Statistical analysis

When normality was confirmed, differences in body weight, gene expression, and cytokine levels between the two groups were evaluated using Student's *t*-test. The results were indicated as mean ± standard deviation (SD) and the statistically significant was set at 5% (*p*-values < 0.05) level.

Results

Effect of SOA on body weight

Mice treated with SOA did not cause body weight alteration at any time point (Fig. 1). At Day 21, the control group had gained 31.21 ± 5.551% body weight compared to Day 1, and the SOA group had gained 35.70 ± 3.507% body weight compared to Day 1.

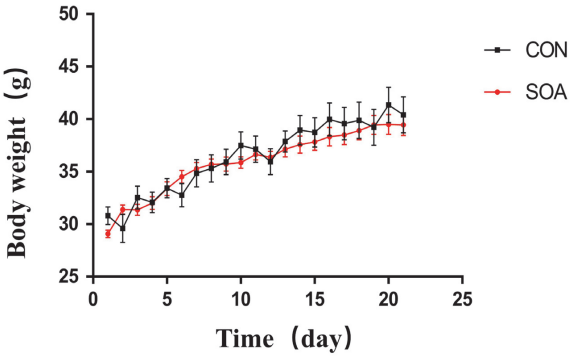

Fig. 1. Body weight of mice in the control and SOA groups

Effect of IFN-γ, IL-2, IL-4, IL-10, and DAO concentration in serum and ileum

SOA treatment significantly increased the serum concentrations of IL-2, IL-10, and IFN-γ compared to the control group ($p < 0.05$; Fig. 2A, 2C, 2D). In contrast, no significant differences were observed in serum IL-4 and DAO levels between the SOA-treated and control groups (Fig. 2B, 2E). Additionally, SOA significantly increased IFN-γ, IL-2, IL-10, and IL-4 concentrations ($p < 0.05$; Fig. 3A, 3B, 3C, and 3D), and decreased DAO concentrations in the ileum (Fig. 3E).

Effect of SOA on the expression of IL-2, IL-4, IL-10, IFN-γ, ZO-1, and Occludin in the ileum

The expression of mRNAs of IL-2, IL-10, and IFN-γ were significantly upregulated by SOA in the ileum ($p < 0.05$; Fig. 4A, 4C, and 4D) whereas IL-4 expressions showed no significant changes between the two groups (Fig. 4B). Besides, the expression levels of ZO-1 and Occludin ($p < 0.05$, Fig. 4E and 4F) were increased in the ileum when the mouse was administrated with 200 mg/kg BW of SOA for 21 days.

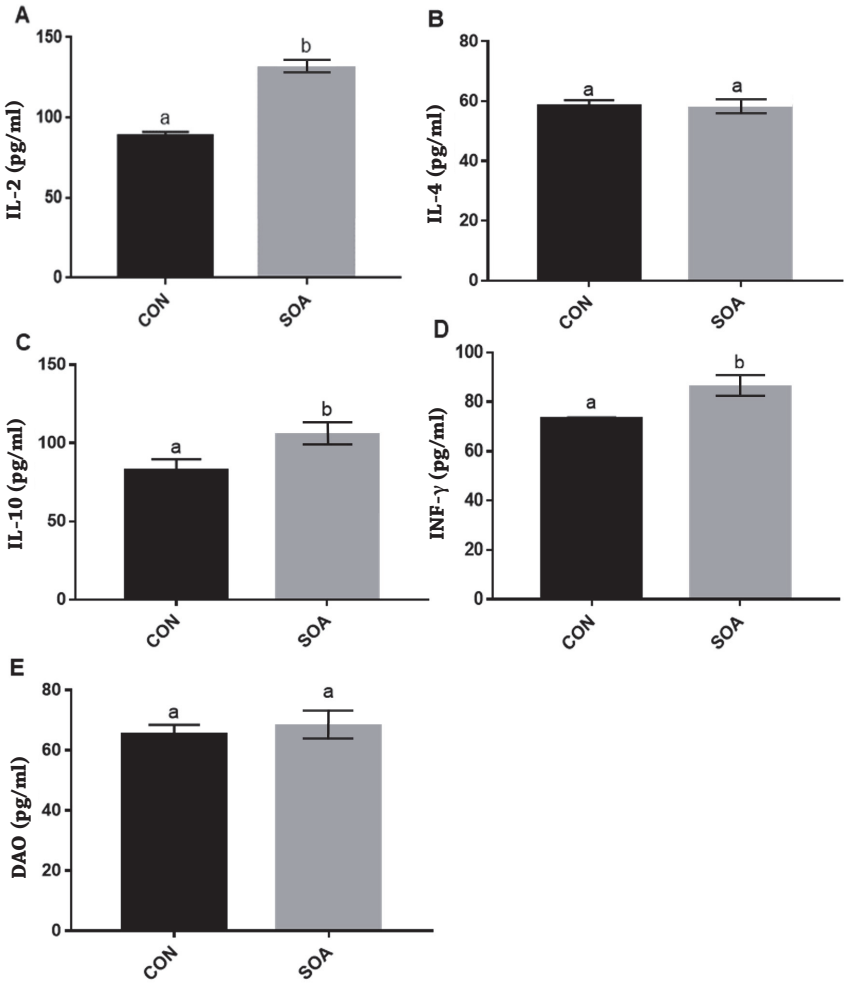

Fig. 2. Effects of SOA treatment on the concentrations of IL-2 (**A**), IL-4 (**B**), IL-10 (**C**), INF-γ (**D**), and DAO (**E**) in serum. Different lowercase letters (a and b) in the same indicator under different treatments represent significant differences ($p < 0.05$).

Effect of SOA on gut microbiota

After pyrosequencing and removing unqualified sequences from 12 cecal content samples, an average of 2,296 ± 3,835 reads per sample were obtained. Based on 97% sequence similarity, the average OTU (Operational Taxonomic Unit) numbers were 305 ± 45 for the control group and 276 ± 40 for the SOA-treated group, indicating that SOA did not increase bacterial OTU numbers. In the sample, the diversity of the communities was reflected by the Simpson and Shannon indices, the richness of the communities was reflected by the Chao and ACE indices, and the detection depth of the samples was reflected by the Coverage index. The Coverage index of the two groups of samples was both greater than 99.9%, and the rarefaction curve of all samples finally tended to be flat, indicating that the sequencing depth covered rare new phylogeny and most of the diversity (Table 2, Fig. 5A). The Chao and ACE richness had not differed significantly between the two groups. The principal component analysis (PCA) and cluster analysis were used to describe the difference between the control group and the SOA group intestinal microbiome. The results showed that there was no significant difference between the two groups (Fig. 5B and 5C). The Venn diagram shows that there were 452 common bacteria between the two groups, as well as their specific bacteria (Fig. 5D).

To explore the differences in the structure of the intestinal microbiota that were altered by SOA feeding, we analyzed their phylum, class, and genus level characteristics. The gut microbiota of the two groups shared a similar overall structure. However, differences in low-abundance taxa (<1%) drove distinct dominant bacterial populations and altered community composition between the groups.

At the phylum level, the predominant phylum of the mice in the CON group and SOA group were *Bacteroidota* and *Bacillota*, and the abundances of them were 55.76% and 39.88% in the CON group, and 58.31% and 35.42% in the SOA group. In the control group, the abundance of *Epsilonbacteraeota* was 1.89%, while in the SOA group it was 3.84%. The abundance of *Proteobacteria* in the SOA group was 1.07%, 0.16% higher than that in the CON group (0.91%) (Fig. 5E). Due to the supplement of SOA, the level of *Deferribacteres* was increased from 0.12% to 0.31%, and the level of *Cyanobacteria* was decreased from 0.39% to 0.10% (data not displayed). It is worth noting that the percentage contribution of *Bacillota* relative to the combined abundance of *Bacillota* and *Bacteroidota* decreased from 71.51% to 60.74%, which suggests that SOA may play an important role in lipid modulatory.

At the class level, *Bacteroidia, Clostridia*, and *Bacilli* were identified as the three dominant bacterial classes. Their abun-

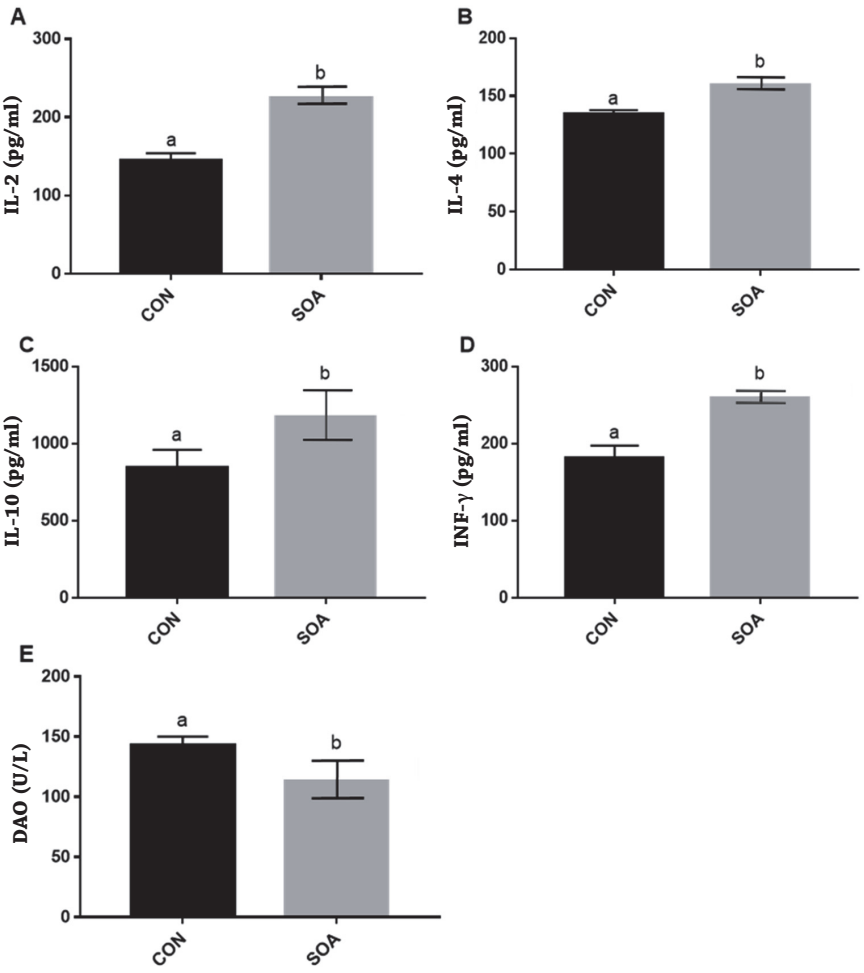

Fig. 3. Effects of SOA treatment on the concentrations of IL-2 (**A**), IL-4 (**B**), IL-10 (**C**), IFN-γ (**D**), and DAO (**E**) in the ileum. Different lowercase letters (a and b) in the same indicator under different treatments represent significant differences ($p < 0.05$).

dances in the control group were 55.76%, 31.40%, and 8.03%, respectively, while in the SOA group, they were 58.31%, 29.78%, and 3.68%, respectively. In the SOA group, *Erysipelotrichia* was increased significantly from 0.45% to 1.96% and *Campylobacteria* was increased from 1.89% to 3.84% (Fig. 5F).

At the genus level, the lower OTU numbers indicated that the SOA group sample could reduce the growth of specific microorganisms. The SOA group significantly increased *Erysipelatoclostridium*, uncultured *Bacteroidales* bacterium, and *Lachnoclostridium*, and significantly decreased *Ruminococcaceae* UCG-010 and *Gordonibacter* (Fig. 5G).

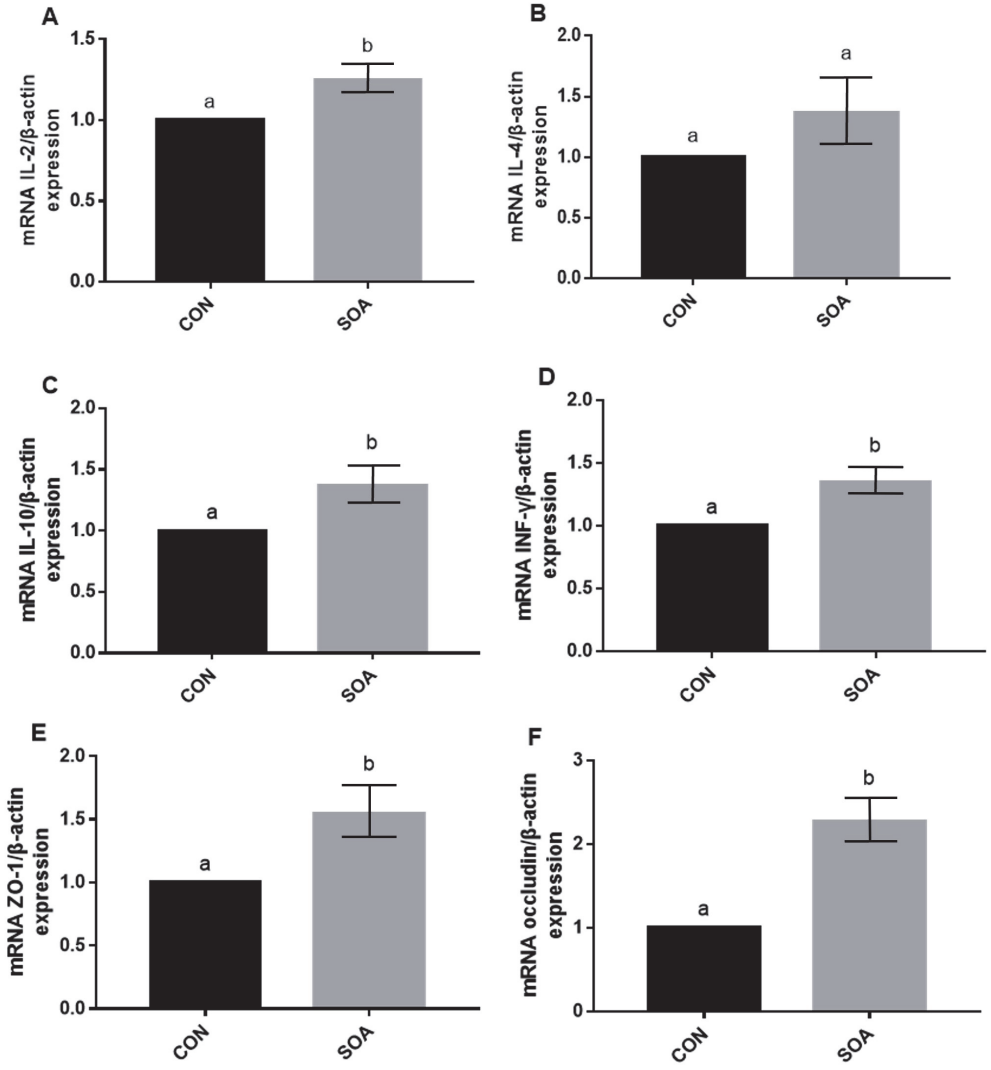

Fig. 4. Effects of SOA treatment on the expression of IL-2 (**A**), IL-4 (**B**), IL-10 (**C**), IFN-γ (**D**), ZO-1 (**E**), and Occludin (**F**) mRNA in the ileum. Different lowercase letters (a and b) in the same indicator under different treatments represent significant differences ($p < 0.05$).

Table 2. Diversity of gut microbiota in mice treated with SOA

Groups	Good's coverage	Richness		Diversity	
		Chao1	ACE	Shannon indices	Simpson indices
CON	0.999852 ± 0.000152	306 ± 46	306 ± 46	6.88 ± 0.37	0.98 ± 0.007
SOA	0.999891 ± 0.000080	277 ± 40	277 ± 40	6.55 ± 0.42	0.97 ± 0.017

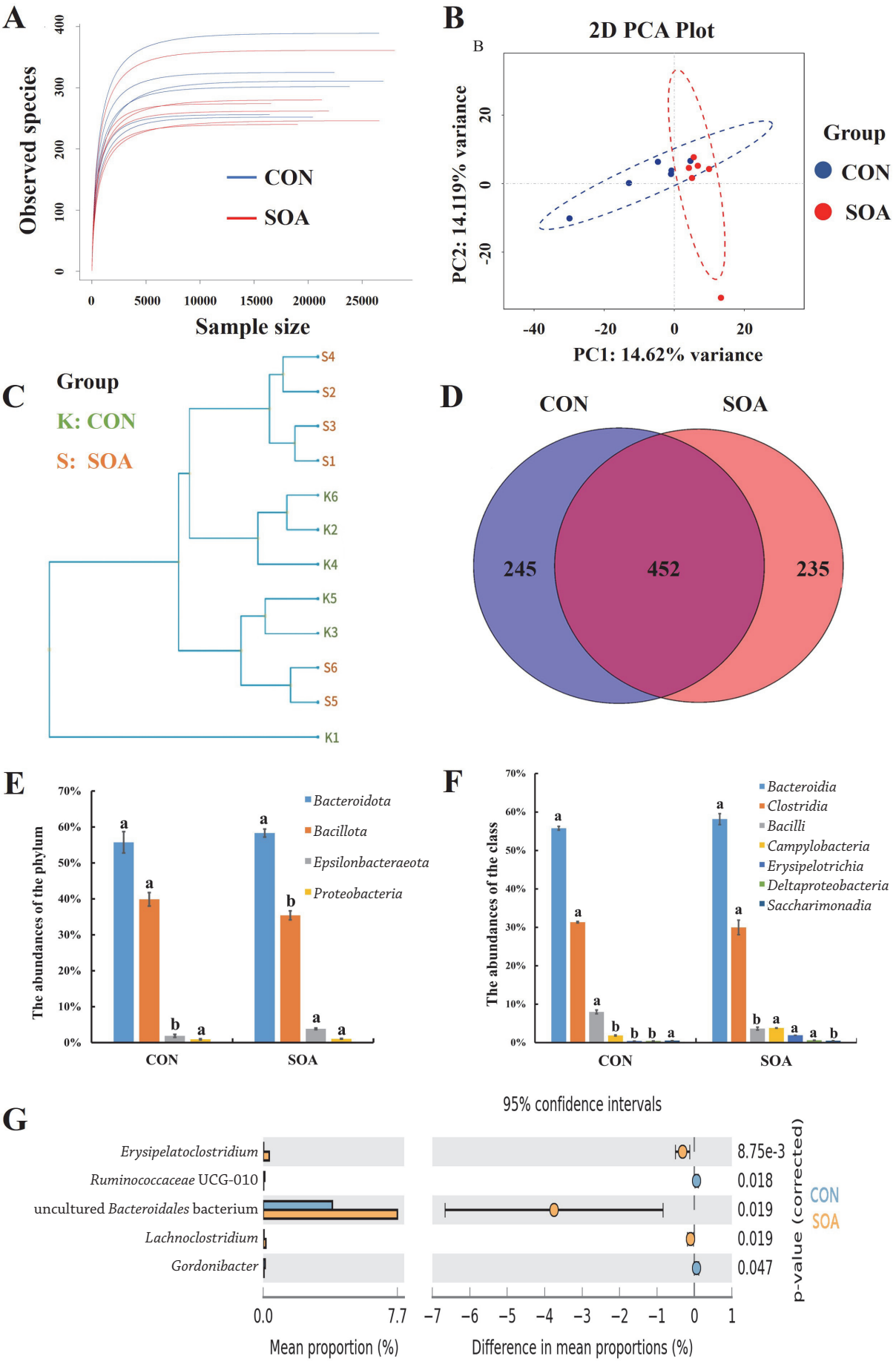
A
Observed species
Sample size
CON
SOA
B
2D PCA Plot
PC2: 14.119% variance
PC1: 14.62% variance
Group
CON
SOA
C
Group
K: CON
S: SOA
S4
S2
S3
S1
K6
K2
K4
K5
K3
S6
S5
K1
D
CON
SOA
245
452
235
E
The abundances of the phylum
Bacteroidota
Bacillota
Epsilonbacteraeota
Proteobacteria
CON
SOA
F
The abundances of the class
Bacteroidia
Clostridia
Bacilli
Campylobacteria
Erysipelotrichia
Deltaproteobacteria
Saccharimonadia
CON
SOA
G
95% confidence intervals
Erysipelatoclostridium
Ruminococcaceae UCG-010
uncultured Bacteroidales bacterium
Lachnoclostridium
Gordonibacter
8.75e-3
0.018
0.019
0.019
0.047
p-value (corrected)
CON
SOA
Mean proportion (%)
Difference in mean proportions (%)

Fig. 5. SOA alters the gut microbiota composition in mice. (**A**) Rarefaction curve. (**B**) PCA clustering analysis. (**C**) Hierarchical clustering analysis of the sample showed differences in the CON group and SOA group. (**D**) Venn diagrams. (**E**) Distribution of gut microbiota at the phylum level. (**F**) Distribution of gut microbiota at the class level. (**G**) Significantly different in the level of genus. (**H**) Heatmap in the level of genus.

Discussion

The intestinal barrier maintains the homeostasis by preventing unexpected movement of antigen molecules and microorganisms from the lumen of the gastrointestinal tract, while allowing digested products and water to enter the body. As a new type of functional food, Selenium polysaccharides have gained growing interest as they have better biological activities and lower toxicity than single polysaccharides or inorganic selenium. Selenium-nanoparticles-loaded chitosan/chitooligosaccharide microparticles have shown their antioxidant potential (Bai et al., 2020). However, there is a lack of relevant studies to focus on the effects of seleno-chitooligosaccharide on intestinal barrier function. This study provided evidence that SOA modulated the mechanical, biological, and immunological barrier in mice. These findings support the potential of SOA as a functional food to improve intestinal barrier function.

The mechanical barrier, primarily composed of epithelial cells and intercellular junctions, serves as the primary defense against pathogen entry. This function is regulated by intricate transcriptional and epigenetic processes (Smith et al., 2017). Disruption of tight junctions in the intestine can lead to heightened permeability to toxins, allergens, and pathogens, contributing to the pathogenesis of gastrointestinal diseases such as celiac disease and inflammatory bowel disease (Tong et al., 2013). Occludin, a key structural and functional component of tight junctions, is essential for their assembly and maintenance (Al-Sadi et al., 2011; Rao, 2009). Zonula occludens-1 (ZO-1) is critical in regulating the pore pathway by bridging Claudin-2 to Occludin (Kuo et al., 2021). Reduced ZO-1 and Occludin expression increases intestinal permeability and induces intestinal diseases (Shin and Kim, 2018). In this study, the supplementation of SOA increased the expression level of ZO-1 and Occludin in mouse ileum, indicating that SOA treatment may decrease tight junction permeability.

DAO is an intracellular enzyme secreted by intestinal mucosal epithelial cells of mammals. Located in the cytoplasm of villus cells at the upper layer of intestinal mucosa of mammals, DAO can convert protoamine into corresponding aldehydes, hydrogen peroxide, and ammonia (Xu et al., 2014). The increase of DAO level in plasma is mainly due to intestinal mucosal injury or increased permeability, which leads to the release of DAO from intestinal cells into the intestinal lumen, and then into lymphatic vessels and blood vessels through the cellular space, and finally into the blood circulation. Therefore, the lev-

el of DAO can reflect the integrity of the intestinal mechanical barrier and the degree of its damage (Hou et al., 2012) – which is an excellent sign of impaired intestinal defense function (Gu et al., 2016). In this study, SOA significantly reduced the level of DAO in the mouse ileum, indicating that SOA could reduce the permeability of the intestine, thereby enhancing the mechanical barrier function of the intestinal mucosa.

The immunological barrier is composed of intestinal lymphoid tissue and intestinal plasma cell-secreted antibodies, in which lymphoid tissues prevent cellular damage from pathogenic antigens through cellular and humoral immunity. Lymphocytes are a major component of the immune system and regulate the intestinal barrier function by secreting various cytokines or producing different cytotoxic effects (Gronke et al., 2017). IL-2, IL-4, IL-10 are all members of interleukin family and have a multi-effect role in the immune response. The source of IL-4 may be basophils (Sokol et al., 2009), NKT cells, and CD4 T cells. IL-4 promotes cell proliferation, survival, and induces naive T cells to proliferate into Th2 cells (that then secrete more IL-4), and enhances communication with Th1 and Th2 cells (Elfarra et al., 2017). It is a glycoprotein containing 113 amino acid residues. Target cells of IL-2 include T cells, NK cells, B cells, and monocyte-macrophages. IL-2 plays an important role in the body's immune response and antiviral infection. IL-10 is mainly produced by Th2 cells and monocyte macrophages. IL-10 promotes intestinal homeostasis – it can inhibit the production of inflammatory cytokines by activated T cells, especially the production of IL-2 and IFN-γ by Th1 cells (Kayama et al., 2012). Th1 cells secrete IFN-γ and play a role in regulating cellular immunity. The alteration of IL-4, IL-2, IL-10, and IFN-γ in the serum and ileum of SOA-fed mice may change the balance of Th1/Th2, thereby exerting a regulatory effect on immune function. The results showed that SOA increased the level of IL-2, IL-10, and IFN-γ in serum and ileum, and improved the production of IL-4 in the ileum. Based on the results, SOA has the potential to regulate intestinal immune function.

The intestinal microbiome is composed of 100 trillion microorganisms, which form the intestinal biological barrier and affect host physiology and health. Many studies have shown that diet plays a vital role in regulating the intestinal microbial composition and maintaining intestinal homeostasis (Ramberg et al., 2010). Most intestinal microbiota data have focused on changes at the phylum level, especially *Bacillota* and *Bacteroidota*. *Bacillota* and *Bacteroidota* represent the two largest phyla in the human and mouse microflora, and the ratio changes in these phyla have been associated with many disease states (Turnbaugh et al., 2008), such as diabetes and obesity (Singer-Englar et al., 2019). In this study, SOA significantly decreased the abundance of *Bacillota* and reduced the percentage contribution of *Bacillota* relative to the combined abundance of *Bacillota* and *Bacteroidota*. Studies have shown that changes in the diet of mice can promote changes in *Bacillota* abundance. Mice fed a high-fat/high-sugar diet have a higher relative abundance of *Bacillota* than mice fed a standard low-fat/high-polysaccharide diet (Chang et al., 2015). A higher amount of *Bacillota* was also associated with more adiposity and body weight in mice (Turnbaugh et al., 2008). When the microbiome of obese mice with higher *Bacillota* abundance was transplanted into the intestines of germ-free mice, the germ-free mice obtained a significant amount of fat compared with those transplanted with the microbiome of lean mice with lower *Bacillota* abundance (Million et al., 2013). Among the gastrointestinal microbiota, *Bacteroidota* has a very broad metabolic potential and is considered to be one of the most stable parts of the gastrointestinal microbiota. In some cases, the reduced abundance of *Bacteroides* is associated with obesity. This bacterial group seems to be enriched in patients with irritable bowel syndrome (Pittayanon et al., 2019) and is involved in type 1 and type 2 diabetes (Rajilić-Stojanović and de Vos, 2014). It has been reported that intestinal microbiota associated with metabolic abnormalities (such as obesity) is characterized by an increase in the abundance of *Bacillota* within these two major phyla (*Bacillota* and *Bacteroidota*), an increase in endotoxin-producing *Proteobacteria*, and a decrease in immuno-homeostatic bacterial species. Our study showed that SOA supplement reduced the contribution of *Bacillota* relative to the combined abundance of *Bacillota* and *Bacteroidota*.

According to a report by Gu et al. (2017), in the streptozotocin-induced diabetic mouse model, the abundance and composition of the mouse intestinal microbiota were altered. Among them, the abundance of *Deferribacteres* was reduced, but after the treatment of the aerial parts of *Salvia miltiorrhiza* Bge, the abundance of *Deferribacteres* was increased. Our results showed that compared with the control group, SOA treatment significantly increased the level of *Deferribacteres*, indicating that SOA could regulate *Deferribacteres* in the intestinal microflora and could provide a reference for the improvement and treatment of diabetes.

In addition, SOA significantly increases the relative abundance of uncultured *Bacteroidales* bacterium and *Bacteroides*. These bacteria can produce short-chain fatty acids (SCFA) by fermentation of polysaccharides. SCFA can regulate the function of the intestine, liver, and adipose tissue, which can effectively prevent high fat diet-induced obesity, and improve insulin resistance, dyslipidemia, and inflammation (Canfora et al., 2015).

Erysipelatoclostridium, Lachnoclostridium, and *Ruminococcaceae* are members of *Bacillota*. Our results showed that SOA significantly increased the relative abundance of *Erysipelatoclostridium* and *Lachnoclostridium*, but significantly reduced the relative abundance of *Ruminococcaceae* UCG-010. Studies have reported that in the intestinal microbiota of patients with inflammatory bowel disease, the abundance of *Lachnoclostridium* was significantly reduced, on account of SCFA production (Wang et al., 2020). In summary, this study indicated that SOA can modulate the gut microbiota and strengthen the intestinal barrier function. Moreover, our findings suggest that SOA has the potential to serve as a beneficial substance for preventing intestinal diseases and enhancing intestinal function.

Future investigations should integrate spatial proteomics and subcellular fractionation approaches to resolve region-specific and compartmentalized effects of SOA on tight junction dynamics, thereby complementing the transcriptional and functional metrics reported herein.

While these findings highlight the therapeutic potential of SOA in intestinal barrier modulation, there are still some limitations. Future studies should prioritize: (1) sex-specific responses to SOA, given the modulation of selenoprotein expression of estrogen; (2) dose-escalation trials to define toxicity thresholds; and (3) clinical validation in selenium-deficient cohorts with barrier dysfunction.

Although selenopolysaccharides have an improving effect on the intestinal barrier, when taking selenium polysaccharides, be aware of risks like selenium overdose, which can cause nausea, vomiting, hair loss, and nail issues. It may interfere with medications and isn't for everyone, particularly those with kidney or thyroid issues. It should be used under a doctor's guidance and blood selenium should be monitored to avoid long-term health issues.

Conclusion

In the present study, a 21-day SOA treatment was administered to ICR mice to explore its function. SOA treatment effectively regulated the levels of inflammatory cytokines, the expression of ZO-1 and Occludin, and the abundance of gut microbiota. Therefore, SOA possesses the ability to improve the intestinal barrier and provides a new possibility in the prevention and treatment of intestinal disease.

Ethics statement

All the animal procedures were approved by the Committee on the Ethics of Animal Experiments of Zhejiang Ocean University [Approval No. SYXK-(ZHE)2019-0031, Permission No. 2023031] and complied with the Laboratory animal – Guideline for ethical review of animal welfare (China).

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Ethical aspects and conflict of interest

The authors have no conflict of interest to declare.

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