RESEARCH ARTICLE

The alleviating effect of polysaccharide extract on intestinal inflammation in mice varies with the sizes and textural properties of shiitake mushrooms

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Intestinal inflammation can trigger gut microbiota imbalance and further inflammatory responses, posing a significant threat to human health. This study aimed to reveal the differences in polysaccharide content among various dried shiitake mushrooms and their effects on intestinal inflammation regulation. Using the phenol-sulfuric acid method, the study found that, under the same diameter conditions, camellia mushrooms had higher crude polysaccharide content than smooth mushrooms with a maximum of 460.5 μg/mL in 2.0-cm-diameter camellia mushrooms. HPLC analysis showed that, while the component characteristics of crude polysaccharides in different-quality mushrooms were generally similar, small-diameter mushrooms lacked distinct single peaks at 11 minutes, indicating that mushroom diameter significantly impacted polysaccharide components. Notably, both crude polysaccharides could down-regulate inflammatory factors like IL-6, IL-1β, and TNF-α in intestinal tissues with camellia mushroom polysaccharides showing better anti-inflammatory effects. They could also suppress the proliferation of pathogenic bacteria like *Salmonella*, thereby improving the gut microbiota of mice. The results demonstrated that both crude polysaccharides probably alleviated intestinal inflammation by repairing the intestinal barrier and inhibiting the activation of the TLR4/MyD88 pathway. This research established the basis for evaluating the quality of dried shiitake mushrooms by polysaccharides and revealed their differences in alleviating intestinal inflammation and regulating gut microbiota dysbiosis, promoting the potential development of high-quality polysaccharides in pharmacological applications.

Keywords: intestinal inflammation; polysaccharides; Shiitake mushrooms; inflammatory factors; gut microbiota.

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Introduction

Acute enteritis, the most common disease of the intestinal tract, is usually caused by bacterial, viral, or fungal infections and directly manifests as acute inflammation of the intestinal mucosa. It is also accompanied by discomfort such as abdominal pain, diarrhea, fever, nausea, and vomiting [1]. In severe cases, complications such as dehydration, electrolyte imbalance, and shock may also occur. Epidemiological data show that more than 179 million people each year seek medical treatment for acute enteritis worldwide with approximately 600,000 hospitalizations and

nearly 5,000 deaths, posing a serious threat to public health [2]. In recent years, the pathogenesis of acute enteritis has been closely linked to imbalances in the gut microbiota in addition to intestinal inflammation, which is involved in the host invasion defense response [3]. The gut microbiota is the core of human intestinal microecology and consists of the intestinal microbiota, intestinal epithelial cells, and the intestinal mucosal immune system. Pathogenic infections, food, and tissue damage can all induce destruction of the intestinal barrier, thereby causing damage to small intestinal villus epithelial cells, reducing their absorption capacity and the release of large amounts of inflammatory mediators, further exacerbating the disruption of microbial homeostasis [4, 5]. Since it is caused mainly by pathogenic bacteria such as *Escherichia coli* and *Salmonella*, the treatment of acute enteritis in clinical practice mostly involves the use of antibiotic drugs such as fluoroquinolones and macrolides. However, the frequent abuse of antibiotics targeting acute enteritis also leads to an imbalance in the intestinal environment and the emergence of intestinal drug-resistant bacteria [6]. Therefore, reducing intestinal inflammation and maintaining the balance of microbial homeostasis are highly important for the clinical treatment of acute enteritis.

Shiitake mushrooms, also known as Lentinula edodes, are the most consumed and cultivated mushrooms worldwide because of their unique taste, flavor, nutritional value, and medicinal properties. As a source of various nutrients and bioactive substances, their fruiting bodies and mycelia contain 71% carbohydrates, 18.64% protein, and 4.8% fat, as well as various vitamins and mineral salts [7, 8]. In recent years, their unique medicinal components, especially polysaccharides, have gradually attracted attention [9]. Numerous studies have demonstrated that polysaccharides are the main active components of shiitake mushrooms that exert multiple pharmacological effects such as anti-tumor, anti-bacterial, anti-viral, anti-oxidation, and immune regulatory effects [10, 11]. Polysaccharides have been shown to have potential application value in preventing and alleviating gastrointestinal inflammation by regulating the gut microbiota and inhibiting the secretion of inflammatory factors [12]. Yang *et al*. demonstrated that lentinan supplementation, an active component from shiitake mushrooms, could improve gut microbiota dysbiosis, steatohepatitis, and the NF-κB–PTP1B–Akt–GSK3β (inflammation–insulin) signaling pathway in the liver, suggesting that it could be a plausible strategy for treating nonalcoholic fatty liver disease (NAFLD) [13]. However, differences in cultivation time, substrate nutrition, temperature, light, and humidity during the cultivation process of shiitake mushrooms directly affected their quality such as size, texture, curled edges, and color, which in turn led to a wide range of uneven qualities of fresh or dried shiitake mushrooms on the consumer market [14]. This difference in quality is reflected not only in the appearance, taste, and nutritional value of shiitake mushrooms but also in their polysaccharide content, which affects their application in health foods, drug development, and other areas. Hence, the functional differences of crude polysaccharides from shiitake mushrooms of different qualities as well as their effect on acute enteritis remain unclear, seriously hindering the potential development of high-quality polysaccharides for pharmacological applications.

Dried shiitake mushrooms, the main form in circulation on the market, generally have different textures or diameters, and the current classification standards cannot provide consumers with criteria for quality selection based on active medicinal ingredients, promoting a healthy diet. This study aimed to investigate the differences in content and composition of shiitake mushroom polysaccharides with varying sizes and textural properties using the phenol-sulfuric acid method and high-performance liquid chromatography (HPLC) and evaluate their effects on the regulation of intestinal inflammation through quantitative reverse transcription polymerase chain reaction (qRT-

PCR) and 16S rRNA sequencing. The results of this study were highly important for evaluating the quality of shiitake mushrooms, optimizing the extraction process, and broadening the biological functions of polysaccharides.

Materials and methods

Mushrooms and experimental animals

Dried shiitake mushrooms with two distinct textures of camellia and smooth and two diameter sizes of 2.0 cm and 4.0 cm were kindly donated by Dadi Mushroom Industry Co., Ltd. (Zhumadian, Henan, China) and Henan Nanshan Biotechnology Co., Ltd. (Zhumadian, Henan, China). *Salmonella typhimurium* (ATCC 14028) was provided by the School of Biological and Food Processing Engineering, Huanghuai University (Zhumadian, Henan, China). Six-week-old adult Kunming mice were purchased from Zhengzhou University (Zhengzhou, Henan, China) and were housed under a 12-hour light/dark cycle with free access to food and water and allowed to acclimate prior to the experiment. All experimental procedures were approved by the Ethics Committee of Huanghuai University (Zhumadian, Henan, China).

Extraction of crude polysaccharides from shiitake mushrooms

Dried mushrooms of both camellia or smooth textures were pulverized into a fine powder (60 mesh) and defatted using Soxhlet extraction with petroleum ether (1:20 w/v) for 6 hours. A precise 5.0 g powder was mixed with distilled water (1:20 w/v) and extracted three times at 80°C for 90 minutes each. After each extraction, the mixtures were centrifuged at 6,000 g, 4°C, for 15 min. The supernatants were pooled and concentrated under reduced pressure. Polysaccharides were precipitated by adding 80% ethanol (4:1, v/v) and incubating overnight at 4°C followed by centrifugation at 6,000 g for 20 min. The precipitates were dissolved in sterilized deionized water to obtain crude polysaccharide extracts. The polysaccharide content was quantified using the phenol-sulfuric acid method with glucose as the standard. All extractions were performed in three independent replicates for each mushroom quality.

Determination of crude polysaccharide concentration

A series of glucose standard solutions (2 mL) with concentrations ranging from 10 to 60 μg/mL were prepared. Each solution was mixed with 1 mL of 6% phenol solution and 5 mL of concentrated sulfuric acid. After incubation at room temperature for 10 minutes, the mixture was reacted in a water bath at 30°C for 20 minutes and then cooled in cold water to terminate the reaction. The absorbance was measured at 490 nm using a UV1000 UV-Vis spectrophotometer (Tianmei Scientific Instrument Co., Ltd., Shanghai, China). A standard curve was constructed based on the glucose standards for quantitative analysis. Similarly, the absorbance of the crude polysaccharide sample solution was measured, and the polysaccharide concentration was calculated using the established standard curve. All measurements were performed independently in triplicate, and data were presented as the mean ± standard deviation (SD).

HPLC analysis

To analyze differences in polysaccharide composition between dried camellia and smooth shiitake mushrooms, HPLC was performed using a Waters Breeze™ 2 system (Waters Corporation, Milford, MA, USA). Sample solutions were filtered through 0.22 μm syringe filters prior to injection. Separation was achieved using an Agilent ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 μm) (Agilent Technologies, Santa Clara, CA, USA) with a gradient mobile phase consisting of 0.1 M phosphate buffer (pH 6.8) and acetonitrile (83:17 to 70:30) (v/v) at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C, and detection was carried out at 254 nm. Polysaccharide components were identified and quantified by comparing retention times and peak areas against standards. All samples were analyzed in triplicate.

Table 1. Sequences of qRT-PCR primers.

Target	Forward sequence (5'-3')	Reverse sequence (5'-3')
GAPDH	ATG TTT GTG ATG GGT GTG AA	ATG CCA AAG TTG TCA TGG AT
IL-1β	ACT CCT TAG TCC TCG GCC A	CCA TCA GAG GCA AGG AGG AA
IL6	GAG GAT ACC ACT CCC AAC AGA CC	AAG TGC ATC ATC GTT GTT CAT ACA
TNFα	TGA TCC GCG ACG TGG AA	ACC GCC TGG AGT TCT GGA A
ZO-1	GCG AAC AGA AGG AGC GAG AAG AG	GCT TTG CGG GCT GAC TGG AG
Occludin	TGG CTA TGG AGG CGG CTA CGG	AAG GAA GCG ATG AAG CAG AAG GC
TLR4	ATG GCA TGG CTT ACA CCA CC	GAG GCC AAT TTT GTC TCC ACA
MyD88	AGG ACA AAC GCC GGA ACT TTT	GCC GAT AGT CTG TCT GTT CTA GT
Salmonella	GCC ATG CTG TTC GAT GAT	GTT ACC GAT AGC GGG AAA GG

Animal experiment

After the acclimation period, 18 male mice were randomly assigned to normal saline, camellia mushroom, and smooth mushroom groups with 6 animals in each group. From the first day, mice in the experimental groups received oral gavage of crude polysaccharides extracted from camellia or smooth mushrooms at the dosage of 200 mg/kg/day every 12 hours for 7 consecutive days, while the control group received an equivalent volume of normal saline. On the 8th day, all mice were intraperitoneally injected with *Salmonella typhimurium* in sterile PBS at a dose of 5 × 10^7 CFU per mouse. The animals were maintained under consistent environmental conditions and monitored closely for 12 hours post-infection before being sacrificed by cervical dislocation and collecting intestine and spleen tissues. All collected tissues were stored at −80°C for subsequent analysis.

qRT-PCR analysis

To quantify specific gene expression in intestinal and spleen tissues, total RNA was extracted using TRIzol reagent (Vazyme, Nanjing, Jiangsu, China) according to the manufacturer's instructions. The concentration and purity of the isolated RNA were assessed using a B-500 type ultramicro UV-Vis spectrophotometer (Yuanxi Instrument, Shanghai, China). Subsequently, 0.5 - 1 μg of total RNA was reverse-transcribed into cDNA using the HiScript 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, Jiangsu, China). qRT-PCR was performed in a 20 μL reaction mixture containing 1 μL of cDNA, 10 μL of ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, Jiangsu, China), 0.5 μL of each forward and reverse primer (Table 1), and 8 μL of ddH_2O. Amplification was carried out on a LightCycler® 96 System (Roche, Mannheim, Germany) under the program of 95°C for 5 min followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The relative gene expression level was analyzed *via* the comparative method (ΔΔCt), and data were presented as the mean ± SD. Experiments were undertaken independently in triplicate with duplicate real time PCR.

Gut microbiome analysis

Cecum contents from mice were collected in sterile microtubes and stored at −80°C following snap-freezing in liquid nitrogen. Total bacterial genomic DNA was extracted and assessed for quality and concentration using NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and 1% agarose gel electrophoresis, respectively. The V3 - V4 hypervariable region of the bacterial 16S rRNA gene was amplified using PCR with the primers 338F (5'-ACT CCT ACG GGA GGC AGC A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3'). The degenerate bases in the primer sequence 806R are included to accommodate genetic variation across bacterial taxa with H represented A, T, or C, V denoted A, C, or G, and W indicated A or T. The amplification program consisted of 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 45 s, and an additional final extension at 72°C for 7 min. The reactions were carried out using the 2× Taq Plus Master Mix (Dye Plus) kit (Vazyme, Nanjing, Jiangsu, China) on a

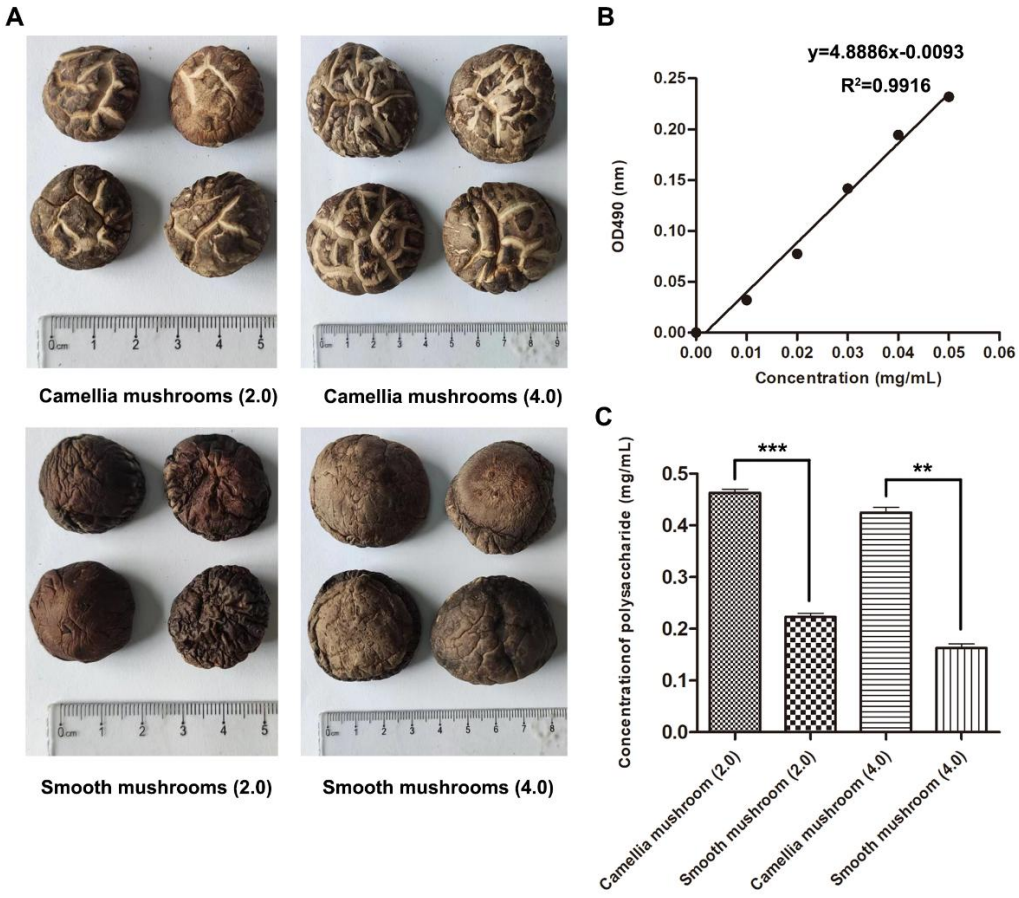

Figure 1. The crude polysaccharide concentrations of different dried shiitake mushrooms. **A.** The appearance of dried shiitake mushrooms with different textures and diameters. **B.** The standard curve for polysaccharide determination. **C.** The polysaccharide concentrations of different shiitake mushrooms measured by phenol-sulfuric acid method.

T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The resulting amplicons were purified, quantified, and normalized. A DNA library was constructed using the TruSeq DNA PCR-Free Sample Prep Kit (Illumina, San Diego, California, USA). High-throughput sequencing was performed on the Illumina HiSeq 4000 platform (Illumina, San Diego, California, USA) using paired-end sequencing with the service provided by Sangon Biotech Co., Ltd. (Shanghai, China).

Statistical analysis

All data were analyzed in GraphPad Prism software (GraphPad Software, Boston, MA, USA) and Graphical representation were performed using Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, California, USA). Significance of statistical analysis was undertaken by Student T test with P values less than 0.05, 0.01, and 0.001 as significant (*), highly significant (**), and very highly significant (***) differences, respectively.

Results

Differences in crude polysaccharide concentrations of diverse shiitake mushrooms

To quantitatively analyze the differences in polysaccharides among shiitake mushrooms with different qualities, four grades of dried mushrooms with two distinct textures (camellia and smooth) and two diameter sizes (2.0 cm or 4.0 cm) were selected (Figure 1A). The standard curve established for polysaccharide quantification demonstrated excellent linearity

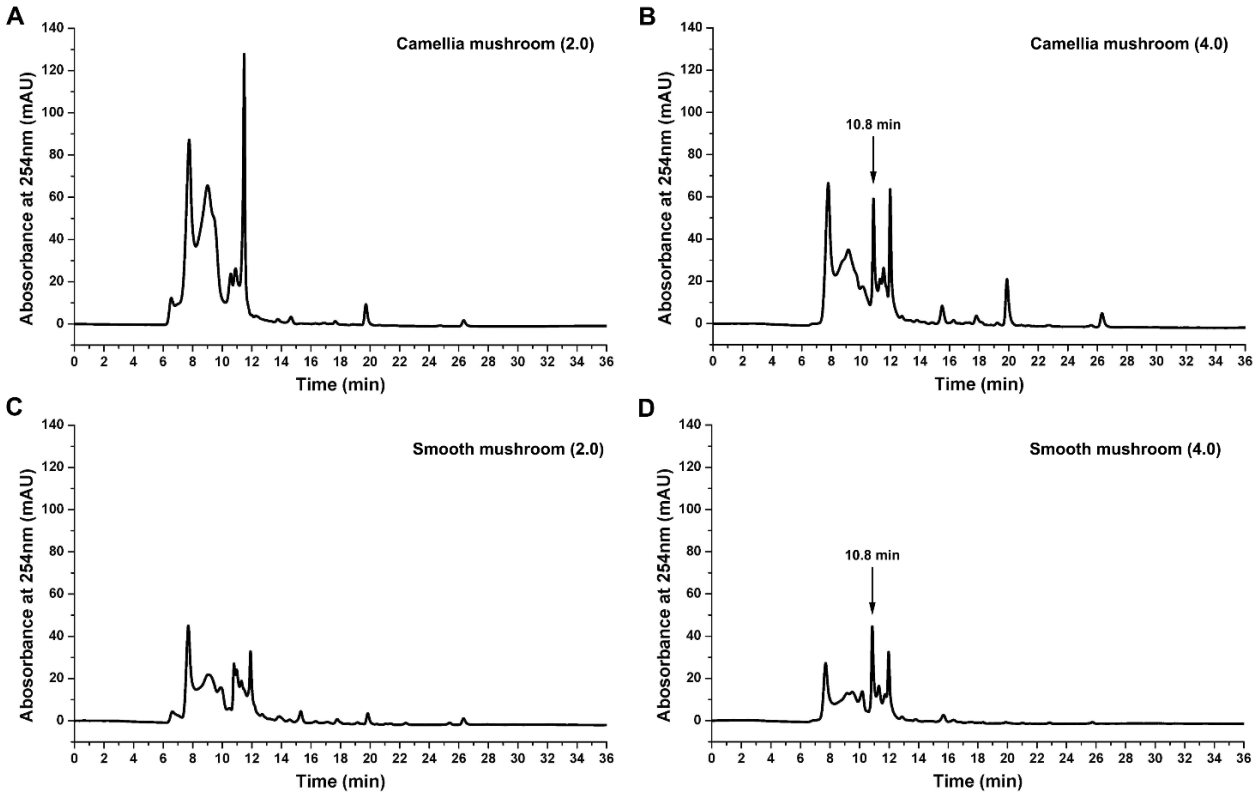

Figure 2. HPLC analysis of four crude polysaccharide components. **A.** camellia mushroom (2.0 cm). **B.** camellia mushroom (4.0 cm). **C.** smooth mushroom (2.0 cm). **D.** smooth mushroom (4.0 cm).

with a coefficient of determination (R^2) of 0.9916, fulfilling the acceptance criterion for a reliable calibration curve (Figure 1B). The concentrations of the crude polysaccharides in different shiitake mushrooms varied significantly with the camellia mushrooms significantly greater than smooth mushrooms. The crude polysaccharide concentration of the 2.0 cm diameter camellia mushroom (camellia (2.0)) was the highest, reaching 460.5 μg/mL (Figure 1C), indicating that the small camellia mushrooms on the market had unique advantages in terms of nutritional and medicinal value. The results suggested that there were significant differences in the internal polysaccharide contents of different shiitake mushrooms, which was highly important for the study of their functional or pharmacological differences.

Differences in crude polysaccharide components compared by HPLC analysis

Four crude polysaccharides were compared using HPLC to further analyze the differences in the crude polysaccharide components of different shiitake mushrooms. The overall HPLC peak heights of the four crude polysaccharides significantly differed with values in the order of camellia mushroom (2.0 cm) > camellia mushroom (4.0 cm) > smooth mushroom (2.0 cm) > smooth mushroom (4.0 cm), which was essentially consistent with the determination results of crude polysaccharide concentrations (Figure 2). Moreover, the HPLC peak shapes of the four crude polysaccharides were essentially the same, while the shiitake mushrooms 4.0 cm showed obvious single peaks at 10.8 minutes after peak emergence but not at shiitake mushrooms 2.0 cm, implying that this monosaccharide component was very likely to exist only in large-diameter shiitake mushrooms (Figure 2). The results once again proved that dried shiitake mushrooms with different sizes

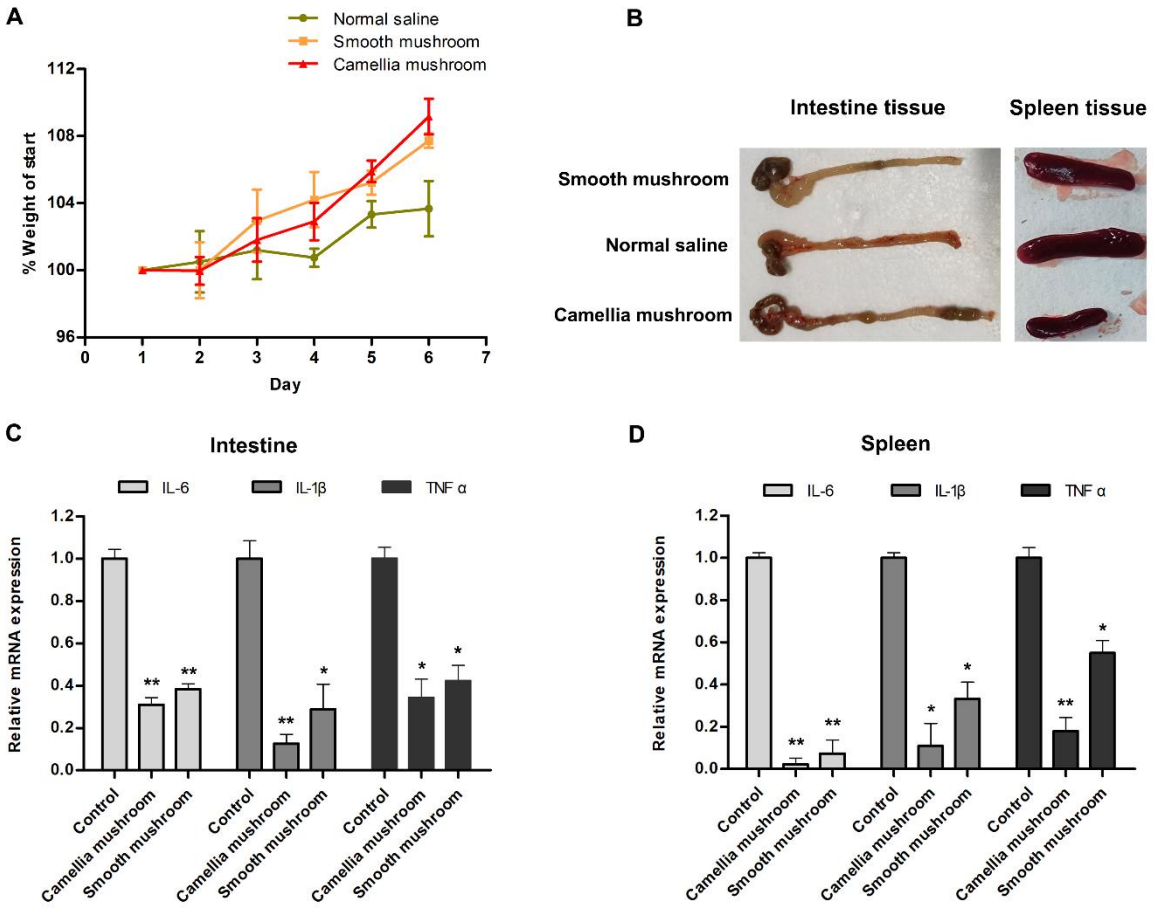

Figure 3. The effects of different crude polysaccharide extracts on inflammation of colon and spleen tissues in mice. **A.** The body weight change curves. **B.** Representative images of colon and spleen tissues from mice in each group. C. The expressions of IL-1β, IL-6, TNF-α in intestine after *Salmonella* infection. **D.** The expressions of IL-1β, IL-6, TNF-α in spleen after *Salmonella* infection.

and textural properties had significantly different polysaccharide concentrations and compositions.

Regulatory effects of different crude polysaccharides on colonic and splenic inflammation in mice with acute enteritis

To compare the functional differences of various crude polysaccharides, the effects of crude polysaccharides from camellia mushroom (2.0 cm) and smooth mushroom (2.0 cm) on gastrointestinal inflammation in mice were investigated. According to the body weight change curve, the weight gain of the mice in the two treatment groups was greater than that in the control group, indicating that both crude polysaccharides improved the growth of the mice (Figure 3A). Bacterial acute enteritis can cause damage to the colonic mucosa, form ulcers, and subsequently lead to colonic atrophy in mice [15]. Therefore, the length of the colon and the size and color of the spleen tissue can directly indicate the degree of inflammation in mouse tissues. After infecting the control group with *Salmonella*, the colonic tissue was significantly morphologically constricted, and the spleen tissue was dark in color and enlarged in shape, indicating that the acute enteritis model was successfully constructed (Figure 3B). In contrast, both crude polysaccharide treatments increased the colon length and reduced the degree of splenomegaly, and the anti-inflammatory effect of the camellia mushroom polysaccharides was more obvious. Furthermore, the regulation of

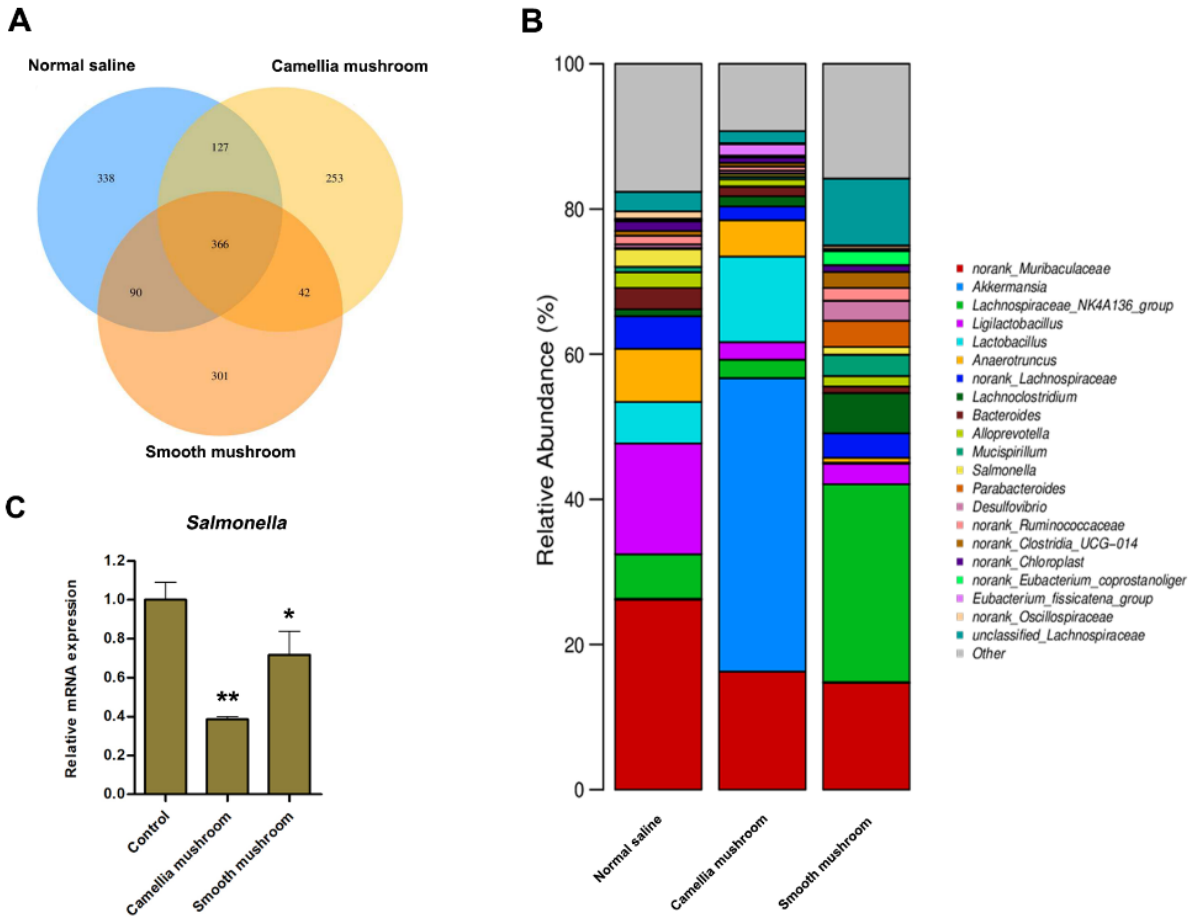

Figure 4. The regulation of two crude polysaccharide extracts on the gut microbial of mice. **A.** The numbers of OTUs in normal saline, camellia mushroom, and smooth mushroom groups. **B.** Relative abundance of Genera levels in gut microbial of three groups. **C.** The relative expression level of *Salmonella* RNA.

inflammatory factors in the intestine and spleen of mice by crude polysaccharides was detected by qPCR. Both crude polysaccharides significantly reduced the expression of IL-1β, IL-6, and TNF-α in the two types of mouse tissues, especially the crude polysaccharides of the camellia mushroom (Figures 3C and 3D). The results indicated that the differential effects of different polysaccharides on alleviating inflammation were expected to provide new ideas for the prevention or treatment of immune-related diseases.

Regulation of different crude polysaccharide extracts on the gut microbial of mice

The results of 16S rRNA gene sequencing showed that a total of 1,517 operational taxonomic units (OTUs) were calculated with 338 and 301 in the normal saline and smooth mushroom groups, respectively. In contrast, 253 OTUs were detected in the camellia mushroom group, indicating that there were still significant differences in the intestinal microbiota of the three groups (Figure 4A). Previous studies demonstrated that the gut microbiota of mice predominantly comprised probiotic genera including *Lnachospiraceae*_NK4A136_9, *Clostridia*_UCG014, and *Lactobacillus*, along with pathogenic bacteria such as *Bacteroides*, *Parabacteroides*, and *Mucispirillum*. Among them, *Lachnospiraceae*_NK4A136_9 is known for its anti-inflammatory properties. *Clostridia*_UCG014 and *Lactobacillus* catabolize carbohydrates into short-chain fatty acids, which

subsequently downregulate inflammation associated with acute enteritis and facilitate the restoration of intestinal barrier integrity [16]. At the genus level, the relative abundance of norank_*Muribaculaceae*, *Bacteroides*, and *Salmonella* in the camellia mushroom group and smooth mushroom group decreased significantly. Moreover, the abundance of *Akkermansia* and *Lactobacillus* was prominent in the camellia mushroom group with *Akkermansia* accounting for approximately 40.41%, indicating that the crude polysaccharides from camellia mushrooms were likely to regulate intestinal inflammation in mice by increasing the abundance of these bacteria (Figure 4B). In addition, the relative abundances of the *Lachnospiraceae*_NK4A136_group and *Clostridia*_UCG014 in the smooth mushroom group also increased significantly, indicating that their improvement in intestinal health was closely related to the high abundance of these bacterial communities. The mRNA expression levels of *Salmonella* in different treatment groups demonstrated that both polysaccharides inhibited the expression of *Salmonella* RNA (Figure 4C). Therefore, the crude polysaccharides from different shiitake mushrooms played important roles in the regulation of gut microbial dysbiosis.

Upregulation of ZO-1/Occludin and inhibition of TLR4/MyD88 by two crude polysaccharides

Previous studies confirmed that the upregulated expression of the tight junction proteins zonula occludens-1 (ZO-1) and Occludin could prevent increased intestinal permeability and repair the intestinal barrier [17]. Meanwhile, TLR4 was a pattern recognition receptor, and its upregulation activated the MyD88-dependent signaling pathway, which led to the production of various inflammatory factors [18]. To elucidate the molecular mechanisms through which the two crude polysaccharides alleviate intestinal inflammation in mice, the expression levels of ZO-1, Occludin, TLR4, and MyD88 were determined by qPCR. Compared with smooth mushrooms, camellia mushroom polysaccharides markedly increased the mRNA expression levels of ZO-1 and Occludin, indicating that they improved barrier function. Intriguingly, both crude polysaccharides could also downregulate the expression of TLR4 and MyD88, indicating that they were likely to reduce intestinal inflammation by inhibiting the TLR4-MyD88 pathway (Figure 5). Crude polysaccharides from different shiitake mushrooms suppressed intestinal inflammation by repairing the intestinal barrier and inhibiting the activation of the TLR4/MyD88 pathway, providing theoretical support for them as novel candidate regulatory agents in the treatment of intestinal inflammation.

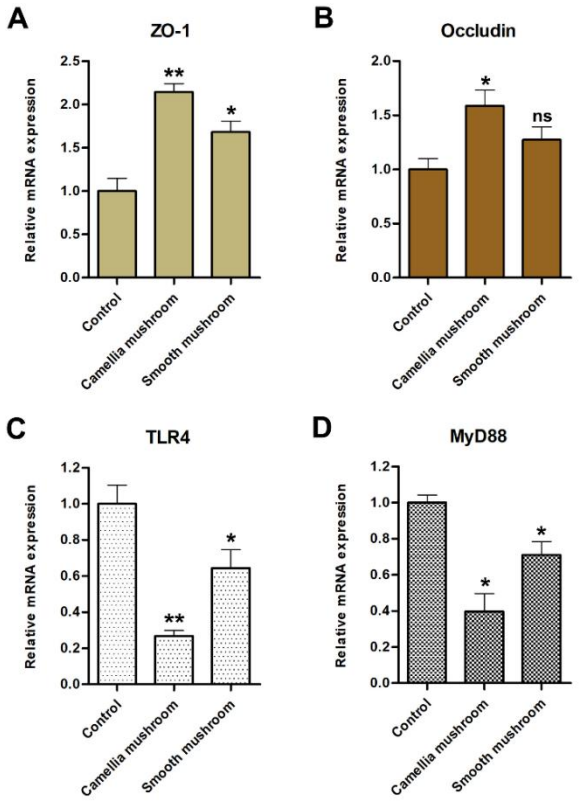

Figure 5. The regulation of two crude polysaccharides on the expression of ZO-1, Occludin, TLR4, and MyD88 in intestine tissue.

Discussion

Shiitake mushrooms, owing to their delicious taste and rich nutritional value, are known as “mountain delicacy” in folk culture. At present,

their industrial development remains largely confined to food-related uses such as mushroom beverages, condiments, health products, and other foods [19, 20]. In the future, focusing on both the nutritional and medicinal attributes is essential for realizing their potential medicinal value and extending the mushroom industry chain, as well as improving the comprehensive utilization of mushrooms. As the primary pharmacologically active substance of shiitake mushrooms, polysaccharides, especially a specific polysaccharide named lentinan (LNT), play important roles in various physiological aspects such as anti-tumor, anti-bacterial, anti-viral, anti-oxidation, and immune regulation [12, 21]. However, differences in cultivation time, substrate nutrition, temperature, light, and humidity directly affect the uneven quality of shiitake mushrooms. Distinct and irregularly distributed white patterns are naturally formed in camellia mushrooms because of the influence of light or temperature during their growth process, whereas the corresponding smooth mushrooms have a smooth surface without obvious patterns, and their color is usually brown or dark brown [8, 22]. The effect of functional differences in crude polysaccharides from shiitake mushrooms of different qualities on various diseases such as acute enteritis remain unclear, seriously hindering the potential development of high-quality polysaccharides for pharmacological applications. By extracting and identifying crude polysaccharides from four types of dried shiitake mushrooms with different sizes and textural properties, this research found that the concentration of crude polysaccharides extracted from small camellia mushrooms was the highest, reaching 460.5 μg/mL, and that the crude polysaccharide content in the camellia mushrooms was significantly greater than that in the smooth mushrooms. The research also measured the concentration of crude polysaccharides in the mushroom stems as a negative control and found that its content was the lowest one at only 151.5 μg/mL, which proved the common phenomenon in the market that mushroom caps were preferred as the main source of nutrition. Due to the excessive development of the lignified fiber structure in the mushroom stem, the dissolution rate and bioavailability of proteins, polysaccharides, and trace elements were significantly decreased, which was consistent with previous report [23]. Although the differences in the components of crude polysaccharide from four dried shiitake mushrooms were analyzed using HPLC in this study, no in-depth analysis of its specific composition was conducted with standard monosaccharide substances or mass spectrometry identification techniques. Guo *et al*. established qualitative and quantitative methods for the analysis of 12 monosaccharides comprising polysaccharides of *Polyporus umbellatus* based on HPLC coupled with electrospray ionization-ion trap-time of flight-mass spectrometry [24]. It is still necessary to further explore the differences in the components of crude polysaccharides among different dried shiitake mushrooms with the help of various quantitative analysis techniques. In addition, fluorescence quantitative PCR revealed that both crude polysaccharides could downregulate the expression of inflammatory factors of IL-1β, IL-6, and TNF-α in the intestinal and splenic tissues of mice. Importantly, the anti-inflammatory efficacy of camellia mushroom polysaccharides was superior to that of smooth mushrooms, suggesting that the pharmacological functions of polysaccharides differ among diverse shiitake mushrooms with different sizes and textural properties. These results were consistent with previous reports that edible fungal polysaccharides could suppress the expression of inflammatory factors and improve the integrity of the intestinal barrier, thereby enhancing the body's immunity [12, 25]. Furthermore, high-throughput sequencing of 16S rRNA revealed that crude polysaccharides from both camellia and smooth mushrooms could increase the abundance of intestinal *Lactobacillus*, *Akkermansia*, *Lachnospiraceae* _NK4A136_group, *Clostridia*_UCG014 and decrease the abundance of norank _*Muribaculaceae*, *Bacteroides*, *Salmonella*, and other pathogenic bacteria. These results indicated that crude polysaccharides in diverse

shiitake mushrooms effectively improved the homeostasis of the intestinal microbiota. Many studies have shown that intestinal microbiota imbalance and inflammation induction can also be manifested as abnormal expression at the molecular level [6, 26]. To elucidate the underlying mechanism, the expression of intestinal tight junction-associated proteins and TLR4-MyD88 pathway genes were analyzed. Notably, the two crude polysaccharides upregulated the mRNA expression of ZO-1 and Occludin and inhibited the expression of TLR4 and MyD88, indicating that they likely suppressed intestinal inflammation by repairing the intestinal barrier and inhibiting the activation of the TLR4/MyD88 pathway.

Conclusion

The results of this study demonstrated that polysaccharides extracted from dried shiitake mushrooms of varying sizes and textural properties exhibited significant differences in both content and composition. Notably, these variations influenced the regulation of inflammatory factors including IL-6, IL-1β, TNF-α and the gut microbiota composition in mouse intestinal tissues. The crude polysaccharides from different shiitake mushrooms suppressed activation of the TLR4/MyD88 pathway, which might contribute to intestinal barrier repair through attenuation of inflammation. However, the underlying molecular mechanisms through which these polysaccharides alleviated intestinal inflammation are still emerging, and further studies are warranted for validation. This research provided new insights into the potential application of quality-differentiated shiitake mushroom polysaccharides in mitigating intestinal inflammation and improving clinical strategies against broad-spectrum antibiotic-associated side effects.

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