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Hericium erinaceus polysaccharides: A potential intervention for antibiotic-associated diarrhea and gut microbiota dysbiosis

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ABSTRACT: In China, the fungus *Hericium erinaceus* is known as a healthy food crop which possesses digestive tract protection functionality. This research focused on the effects of polysaccharides extracted from *H. erinaceus* (WHP) on rats with antibiotic-associated diarrhea (AAD), specifically on the shifts of gut microbiota. WHP was mainly composed of glucose (88.6%), galactose (9.1%), and fucose (2.3%). It decreased the disease activity index and structural damage of the colon in rats with AAD; increased butyrate production and affected the gut microbiota through increase the relative abundance of *Lactobacillus* and *Butyricicoccus*, while decreased that of *Allobaculum* and *Enterococcus*, which closely related to the gut microbiota dysbiosis. This study demonstrates that WHP can ameliorate antibiotic-induced diarrhea by causing the recovery of the colon damage, improving the gut microbiota dysbiosis, and promoting the butyrate production. The results of this study can provide valuable data on how *H. erinaceus* polysaccharides alleviate the damage associated with the intake of antibiotics.

KEYWORDS: *Hericium erinaceus*, polysaccharide, antibiotic-associated diarrhea, gut microbiota, butyrate

INTRODUCTION

Hericium erinaceus, a traditional edible-medicinal fungus in China, is valued for its remarkable medicinal potential, with its fruiting bodies attracting considerable attention in medical, biological, and food science fields due to diverse bioactivities [1, 2]. It is commonly used as a home remedy (e.g., in soups) to ameliorate chronic atrophic gastritis and other digestive tract disorders. As a major bioactive component of *H. erinaceus*, polysaccharides have been extensively investigated for their purification, structural characteristics, and pharmacological properties [1].

Notably, the extraction method of *H. erinaceus* polysaccharides directly influences their bioefficacy. *In vitro* studies demonstrated that citric acid-extracted polysaccharides exhibit more potent antioxidant activity compared to those extracted with water or 0.9% sodium chloride [3]. Polysaccharides from *H. erinaceus* mycelium possess anti-gastritis, anti-ulcerative colitis, hepatoprotective, antioxidant, and anti-gastric ulcer effects [4], and can inhibit hydrogen peroxide-induced apoptosis of gastric mucosal epithelial cells [5]. Fruiting body-derived polysaccharides exert gastroprotective effects by regulating gastric secretions, enhancing antioxidant status, and promoting defensive factor release [6], such as β-glucan (modulating wheat starch digestibility [7]), HP-ES (regulating Th1 immune responses via dendritic cell activation [8]), HECP and HCRP (improving colon health through short-chain fatty acid (SCFA) production [9]), HECP (alleviating colitis-related intestinal mucosal oxidative damage and gut microbiota dysbiosis [10]).

Antibiotics are indispensable for treating infectious diseases, but their overuse or abuse frequently induces antibiotic-associated diarrhea (AAD), gut microbiota imbalance, and other complications that impede recovery [11]. Gut microbiota dysbiosis is closely linked to digestive tract diseases [12], with gastrointestinal tumors, ulcerative colitis, and chronic gastritis consistently associated with altered microbial diversity and composition [13, 14]. While *H. erinaceus* fruiting body polysaccharides have been shown to restore gut microbiota balance in colitis mice by increasing the relative abundance of *Clostridiales*, *Akkermansia*, and *Desulfovibrio* [10], their efficacy in alleviating AAD and antibiotic-induced gut microbiota dysbiosis remains unclear.

Herein, we established an AAD rat model by orally administering lincomycin hydrochloride to investigate the effects of water-soluble polysaccharides from *H. erinaceus* (WHP) on AAD-related phenotypes, focusing on gut microbiota composition, colon histomorphology, and SCFA metabolism. This study aims to provide experimental evidence for the potential application of *H. erinaceus* polysaccharides in mitigating antibiotic-induced adverse effects.

MATERIALS AND METHODS

Materials and reagents

Dried *H. erinaceus* fruiting bodies were purchased from Tonghua (Jilin, China). Lincomycin hydrochloride was obtained from CR Double-Crane Pharmaceuticals Co.,

Ltd. (Beijing, China). The TIANamp STOOL DNA Kit (cat. No. DP328) was purchased from Tiangen Biotech Co., Ltd. (Beijing, China). Acetate, propionate, and butyrate were sourced from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Extraction procedures and physiochemical analysis of *H. erinaceus* polysaccharides

Dried *H. erinaceus* fruiting bodies (500 g) were boiled in 10 l of distilled water for 3 h. Then filtrated with a gauze (100 mesh), and the residue was used to repeat the extracting step above twice. The extracting solution was concentrated to 1.5 l at 60 °C, and centrifuged (2,940×g, 10 min). The supernatant was combined with 6 l anhydrous ethanol for 12 h and centrifuged (2,940×g, 10 min). Dissolved the precipitates in 1 l distilled water, then treated with addition of 4 l anhydrous ethanol. Then dissolved precipitates in 500 ml distilled water, shaken thrice with 500 ml Sevag reagent (chloroform: n-butyl alcohol [v:v] = 1:5) to remove the protein. The water layer was collected and freeze-dried, and WHP were obtained. The monosaccharide composition, uronic acid, and carbohydrate contents were analyzed as previous reports [15, 16]. Protein content was analyzed by NDA 701 Dumas nitrogen analyzer.

Experimental design

Wistar rats (180 ± 20 g) were purchased from Changsheng Laboratory Animal Technology Co., Ltd. (Shenyang, China), they were raised at 22 ± 0.5 °C, 50 ± 5% humidity, and 12 h:12 h light:dark cycles circumstances, with free access to food and water. Rats were randomly divided to four groups (6/group), the control (C) group, AAD (DM) group, natural recovery (NR) group, and WHP treatment (WHP) group after acclimation. The AAD model was established by oral gavage of lincomycin hydrochloride (10 ml/kg) twice daily for 4 days, except for the C group which received saline. After antibiotic treatment, rats in the DM group were anesthetized, and blood samples, cecal contents, and colon samples were collected. Then rats of WHP group were treated WHP (100 mg/kg) twice a day for 7 days, whereas other groups received same amount of physiological saline. The same methods were used to collect the samples as above.

The disease activity index (DAI) was an important indicator to evaluate the severity of inflammatory bowel disease (IBD) [17]. The DAI score is the average of the following three scores: (I) weight loss (0: none; 1: 1%–5%; 2: 5%–10%; 3: 10%–15%; 4: >15%), (II) stool consistency (0: normal; 1 and 2: loose; 3 and 4: diarrhea), (III) stool blood (0: normal; 1: +; 2: ±; 3: ++; 4: >++).

Histological analysis

Colon samples were treated as our previous study for histological observation [18]. The colon was collected and fixed in 10% (v/v) formalin, dehydrated in ethanol, embedded in paraffin, cross-sectioned at a thickness of 4 or 5 µm, stained with hematoxylin and eosin, and then observed under an Olympus BH22 Microscope (Tokyo, Japan).

Microbiota analysis

Deoxyribonucleic acid (DNA) pretreated methods were performed as previously reported [18]. R software (ver. 3.2.0), and the Greengenes database [19] were used to compare the operational taxonomic units. The α diversity of abundance-based coverage estimator (ACE) index was calculated to compare the diversity of gut microbiota [20, 21]. The β diversity was determined using principal component analysis (PCA) [22] to identify microbiota variations. Partial Least Squares Discriminant Analysis (PLS-DA) was used to identify the inter-group differences between species [23]. The abundance of taxa at phylum level among samples or groups was compared [24]. Lefse was used to find the key species based on the linear discriminant analysis [25]. All raw sequences were deposited into the NCBI Sequence Read Archives.

Measurement of SCFAs

The SCFAs were analyzed as previously reported [26]. The cecal content (100 mg) of each rat was weighed and put into a centrifuge tube. Then, 10 µl of 15% ortho-phosphoric acid, 100 µl of adipic acid (50 µg/ml, internal standard) solution and 400 µl of ether were added in sequence. The mixture was vortexed for 1 min and centrifuged at 16,100 g for 10 min at 4 °C. The supernatant was filtered through a 0.45 µm organic-compatible membrane filter for the assay.

In addition, standard solutions of acetate, propionate, and butyrate at different concentrations were prepared in ether. All assays were performed using the Agilent 6890N/5975B GC-MS System, Agilent (Santa Clara, USA). The separation of each compound was achieved with an Agilent HP - INNOWAX capillary column.

The initial oven temperature was 90 °C, held for 3 min. Then, it was increased to 120 °C at 10 °C/min, to 150 °C at 5 °C/min, and finally to 250 °C at 25 °C/min and held for 2 min. The ion source and injection port temperatures were set at 230 °C and 250 °C, respectively. The injected volume was 1 µl. The helium flow rate was 1 ml/min with a 10:1 split ratio. For mass spectroscopy, an electron bombardment ionization (EI) source, full scan, and SIM scanning mode were used, and the electron energy was 70 eV.

Table 1 DAI scores, colon weight, and colon length (mean ± SD, $n = 6$).

Group	DAI	Colon weight (g)	Colon length (cm)
C	0.0 ± 0.0^{c}	1.92 ± 0.27^{a}	12.26 ± 0.95^{a}
DM	3.1 ± 0.4^{a}	1.46 ± 0.23^{b}	10.15 ± 0.82^{b}
NR	2.5 ± 0.4^{a}	1.54 ± 0.33^{b}	10.82 ± 1.15^{b}
WHP	0.7 ± 0.1^{b}	1.86 ± 0.31^{a}	11.97 ± 1.01^{a}

[a–b] Data within a column with different superscripts differed significantly ($p < 0.05$). C, control group; DM, antibiotic-associated diarrhea group; NR, natural recovery group; WHP, *H. erinaceus* polysaccharide-treated group.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5. All data were expressed as means ± standard deviation (SD). Comparisons between groups were performed using the one-way analysis of variance or t-test. Differences were considered significant at $p < 0.05$.

RESULTS

Physiochemical structures of WHP

The yield of WHP was 7.5% (w/w). The concentrations of total carbohydrates were 95.6%, and protein was 1.61%. WHP was composed of glucose (88.6%), galactose (9.1%), and fucose (2.3%).

Normal atatus, DAI scores and colon status of rats

The rats with AAD showed lower food consumption, increased water intake, higher defecation frequency, and diarrhea. Compared with group C, the rats of group DM showed decreased body weight increment (Fig. 1A) but increased water intake (Fig. 1B), suggesting the successful establishment of the AAD rat model. During the model establishment period, body weight gradually increased while water consumption significantly increased (Fig. 1). Compared with physiological saline treatment, the water consumption decreased, body weight increment was increased after treating WHP. WHP could promote the normal status recovery of antibiotic-associated side effects in rats.

The DAI reflected the diarrhea status of the AAD rats. A lower DAI value indicates a better recovery status. As shown in Table 1, WHP decreased the DAI significantly compared with the NR and DM groups. Colon weight and length reflect intestinal injury and recovery in rats. Compared with the C group, the DM group exhibited reduced colon weight and length. Compared with the DM group, the NR group showed partial but non-significant recovery. However, after WHP intervention, both colon length and weight returned to normal levels, indicating a marked improvement in intestinal injury, suggested that WHP obviously alleviated the diarrhea status and gut injury resulting from lincomycin hydrochloride.

Effects of WHP on colon structure

HE staining was performed to observe the pathological and morphological changes of intestinal tissues in each group (Fig. 2). In group C, the intestinal mucosa was structurally intact, with well-arranged and morphologically normal villi and regular crypts. No erosion, epithelial shedding, or significant inflammatory cell infiltration was observed, indicating normal intestinal morphology.

Compared with group C, the DM model group exhibited obvious pathological intestinal damage. Intestinal villi were extensively atrophied, shortened and blunted, with partial villous fusion, lodging and focal exfoliation. The continuity of the intestinal mucosal epithelium was disrupted, accompanied by evident mucosal erosion. Moreover, massive inflammatory cell infiltration was observed in the lamina propria and submucosa, along with tissue congestion and edema, suggesting a severe intestinal inflammatory response. These findings confirmed the successful establishment of the intestinal injury model.

In the NR group, intestinal tissue damage was alleviated to a certain extent through self repair, with improved villus arrangement, restored mucosal integrity, and relieved inflammatory infiltration. However, compared with the C group and the WHP treatment group, the repair effect in the NR group was incomplete. Partial villi presented mild atrophy and apical blunting, and local epithelial repair remained incomplete. A small number of residual inflammatory cells were distributed in the lamina propria, accompanied by slight submucosal edema. These results indicated that intestinal damage could not be fully repaired merely by spontaneous recovery, with persistent low-grade inflammation and incomplete mucosal structural repair.

Noticeable improvements in intestinal histopathology were observed in the WHP group. The intestinal villi were densely and regularly arranged, and the villus height, villus morphology, and crypt structure were restored to nearly normal levels. The mucosal epithelium remained intact without erosion or defect, inflammatory cell infiltration in the lamina propria was largely diminished, and tissue congestion and edema were markedly ameliorated. In conclusion, WHP effectively ameliorates intestinal structural damage and reduces inflammation, with a superior reparative effect compared to NR group.

Effects of WHP on gut microbiota

Diversity of gut microbiota

We used ACE index analysis to identify the differences in the richness and diversity of gut microbiota. It was suggested that the richness significantly decreased in DM group compared with the C group. As expected, the ACE index recovered in NR group and WHP group (Fig. 3A). There were significant differences between rats of C and DM groups, as well as C and NR groups.

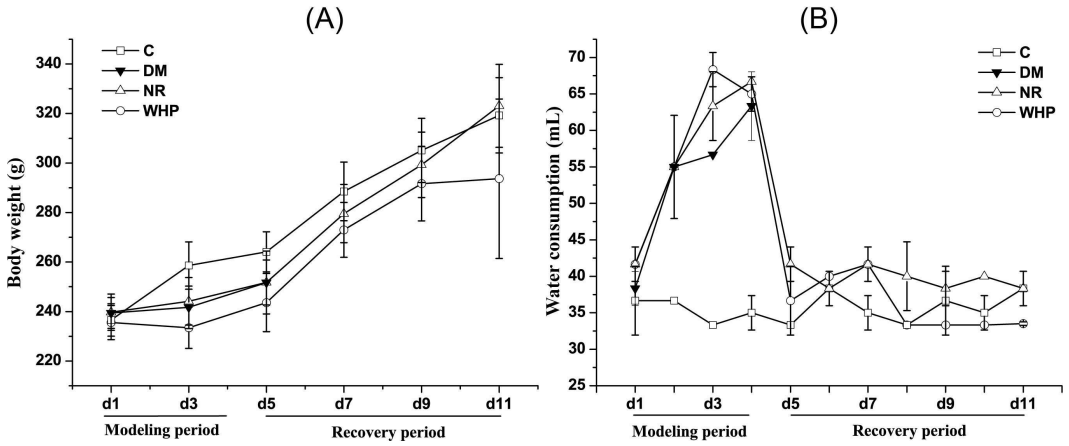

Fig. 1 Effects of WHP on body weight (A) and water consumption (B). C, control group; DM, antibiotic-associated diarrhea group; NR, natural recovery group; WHP, *H. erinaceus* polysaccharide-treated group. Data are expressed as means ± SD. ($n = 6$).

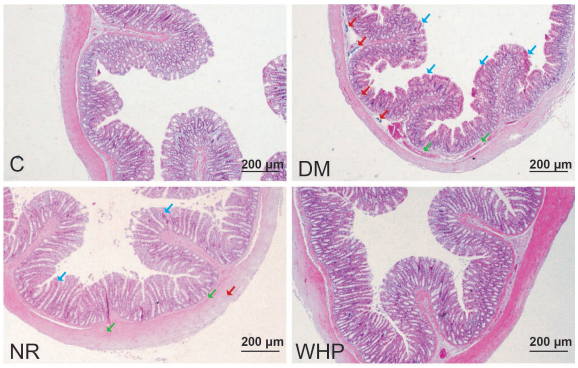

Fig. 2 Colon ultrastructure (magnification, 40×). C, control group; DM, antibiotic-associated diarrhea group; NR, natural recovery group; WHP, *H. erinaceus* polysaccharide-treated group. Red arrows: inflammatory infiltration; green arrows: tissue edema; blue arrows: epithelial shedding and disruption.

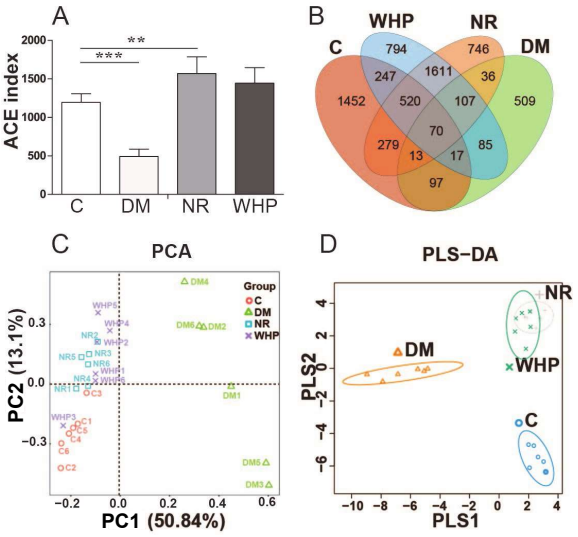

Fig. 3 Difference analysis of the gut microbiota. (A) The ACE index, (B) the Venn analysis, (C) the PCA analysis, (D) the PLS-DA analysis. C, control group; DM, antibiotic-associated diarrhea group; NR, natural recovery group; WHP, *H. erinaceus* polysaccharide-treated group. Data are expressed as means ± SD. ($n = 6$). ** $p < 0.01$; *** $p < 0.001$.

Interestingly, no significant difference were shown between C and WHP groups, suggesting that WHP could improve the recovery of the richness of the gut microbiota nearer to the C group than physiological saline. It was also shown in the Venn analysis (Fig. 3B) that the total and unique Operational Taxonomic Unit (OTU) in groups C, DM, NR, WHP were accordance with the results of ACE index.

PCA and PLS-DA analysis were used to analyze similarities that existed in microbiota between groups. DM group showed great shift on microbiota communities compared with the C group, however, after treatment with physiological saline or WHP, there were some recovery tendencies (Fig. 3C,D). According to PCA, the structure of microflora community reduced which was similar between C and DM groups, suggesting the structural changes after antibiotic treatment. However, the structure of microflora community of NR and WHP groups was similar and close to the C group. PLS-DA results were consistent with PCA, which showed that the microbial communities' structure of each group was relatively independent, and the NR and WHP groups were recovered following antibiotics destruction.

Composition shifts of gut microbiota

C, DM, NR, and WHP groups showed significant changes in the composition and amounts of gut microbial species. The four groups were mainly com-

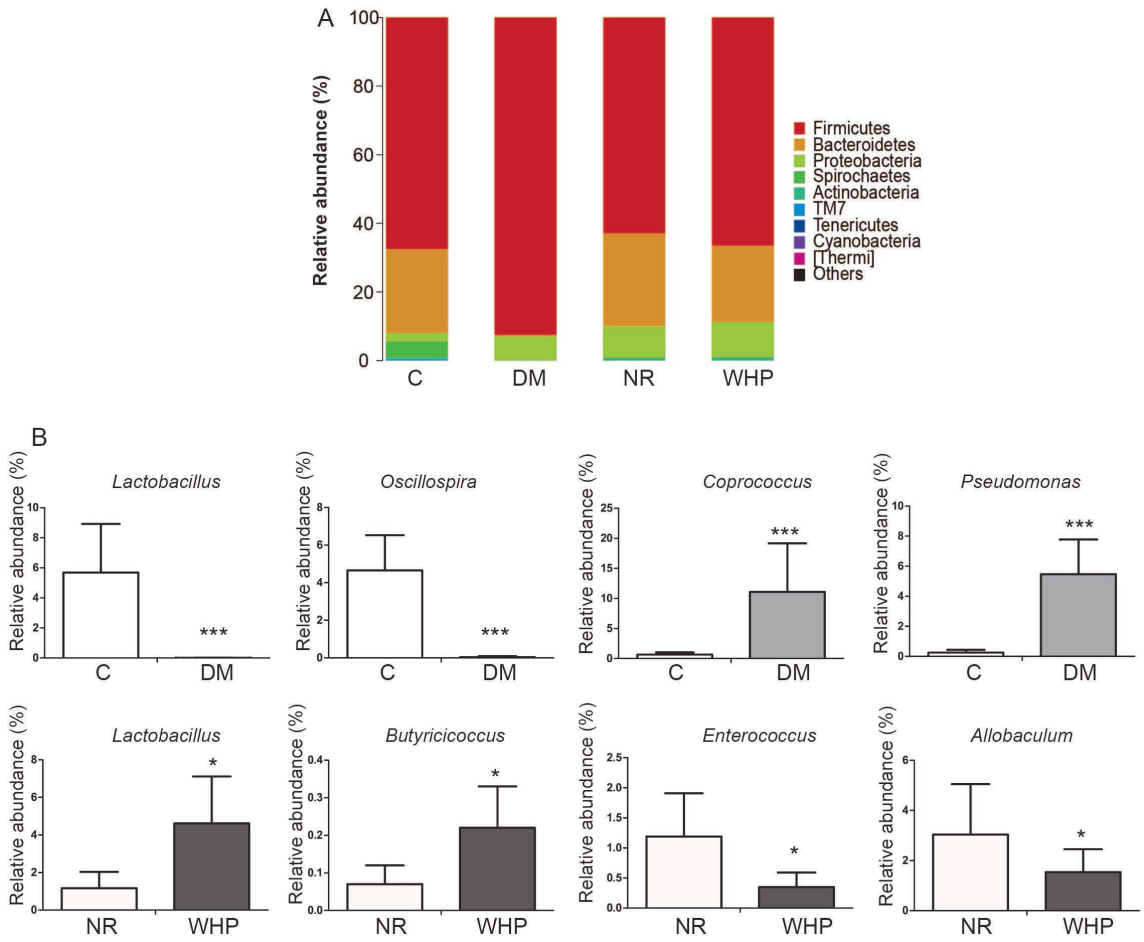

Fig. 4 The composition of gut microbiota. (A) Phyla level; (B) Genus level. C, control group; DM, antibiotic-associated diarrhea group; NR, natural recovery group; WHP, *H. erinaceus* polysaccharide-treated group. Data are expressed as means ± SD. ($n = 6$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

posed of Firmicutes, Bacteroidetes, and Proteobacteria (Fig. 4A) at the phylum level. The relative abundance of Bacteroidetes was significantly decreased in rats of DM group, while Firmicutes and Proteobacteria showed dramatic increase. The results in the composition of the gut microbiota as seen between the DM and C group suggested the successful construction of the AAD model which along with the microbiota dysbiosis. Bacteroidetes and Firmicutes recovered to their normal levels, while Proteobacteria still increased in NR and WHP groups.

The gut microbiota composition showed great shift among the four groups at the genus level (Fig. 4B). The relative abundances of *Coprococcus* and *Pseudomonas* significantly increased, while *Lactobacillus* and *Oscillospira* decreased in the DM group compared with C group, respectively. In the NR and WHP groups, the relative abundance of *Coprococcus*, *Lactobacillus*, and *Oscillospira* were seen to have recovered to the level of C Group. Compared with the NR group, the levels of *Lactobacillus* and *Butyricicoccus* significantly increased, while *Enterococcus* and *Allobaculum* decreased in WHP group (Fig. 4B). Although the abundance of *Lactobacillus* was increased both in NR and WHP groups compared with DM group, WHP treatment was more efficient than that of physiological saline.

SCFAs production

Acetate, propionate, and butyrate were measured to investigate the effects of WHP on microbial metabolites (Fig. 5). Caeca contents of DM group revealed decreased concentrations of acetate, propionate, and butyrate compared with that of C group. The NR and WHP groups exhibited significant recovery in acetate and propionate production. The level of butyrate significantly increased in the WHP group compared with NR group ($p < 0.05$), while acetate and propionate did not show obvious changes.

DISCUSSION

Diarrhea and colon structure destruction have been the common side effects of excessive antibiotics intake.

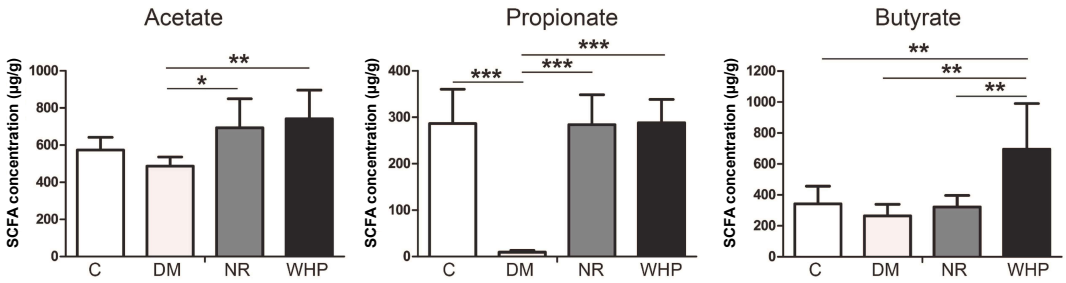

Fig. 5 SCFA in caecal contents. C, control group; DM, antibiotic-associated diarrhea group; NR, natural recovery group; WHP, *H. erinaceus* polysaccharide-treated group. Data are expressed as means ± SD. ($n = 6$). * $p < 0.05$; ** $p < 0.01$.

Our results showed that following antibiotic treatment, the intestinal structure exhibited some capacity for self-repair, however, the intervention of WHP could enhance the effects above, decrease the inflammatory infiltration, and protect the colon structure faster and more effectively, as well as the sufficient improvement of the diarrhea status.

According to the diversity analysis of the gut microbiota, both NR and WHP could recover from the damage caused by antibiotics to a certain extent. But WHP was more orderly than NR in the recovery period; the abundance and diversity of gut microbiota were closer to that of normal mice, especially the diversity index based on ACE. The composition of gut microbiota in NR group had little change compared with WHP at the phylum level, mainly because of the phylum level is a broad taxonomic rank, and each contains many members. However, at the genus level, the disruption caused by antibiotics not only reduces the number of bacteria but also changes their composition. The most obvious was that WHP could promote the relative abundance of *Lactobacillus* and *Butyricicoccus*, while a decrease in *Allobaculum* and *Enterococcus* was observed. *Lactobacillus* was recognized as beneficial bacterium, which has a positive role in promoting human health, such as conquering diarrhea infection [27, 28]. The *Butyricicoccus* is a genus found in many probiotics, which has always displayed lower level in fecal samples of IBD patients [29]. *Butyricicoccus* was related to the production of butyrate, a type of SCFAs that showed beneficial effects to the host. *Allobaculum* and *Enterococcus* were related with gut microbiota dysbiosis. Although *Enterococcus* is no longer considered to be a typical food borne pathogen, its presence often raises health concerns. At the same time, some strains in the *Enterococcus* genus were proved to possess resistance to many antibiotics, and play an important role in infections, such as nosocomial infections, bacteremia, endocarditis [30]. Therefore, the reduction of *Enterococcus* by WHP appears beneficial. So, the improving effects of WHP on the AAD might be correlated with the ability to adjust the relative abundance of *Lactobacillus*, *Butyricicoccus*, *Allobaculum*, and *Enterococcus*, which are all associated with the health of the gastrointestinal tract.

To research the different influences of the various polysaccharides on AAD, we used the same method and compared the results with the polysaccharides derived from *Schisandra chinensis* (WSP) [26] and *Astragalus membranaceus* (WAP) [31]. When compared with physiological saline treatment, WSP could promote the relative abundance of *Intestinibacter*, *Blautia*, and *Lachnospiraceae*-UCG-008, but reduced that of *Ruminococcaceae*-UCG-014, *Ruminococcus*-1, and *Erysipelatoclostridium*. WAP could increase the relative abundance of *Pseudomonas*, but decreased *Allobaculum* and *Coprococcus* abundance at the genus level. At the same time, there are some similarities in the effects on some species. For example, both WHP and WAP can reduce the abundance of *Allobaculum*. These results suggest that different sources of polysaccharides showed various effects on gut microbiota, which may be related to the differences of monosaccharide composition, molecular weight, length of the main chain, space conformation and so on.

It has been reported [10] that polysaccharides from the fungus *H. erinaceus* could improve the relative abundance of *Clostridiales*, *Akkermansia* and *Desulfovibrio* which would in turn caused a balance in the gut microbiota in mice with colitis. However, in our study, WHP was not found to influence the abundance of these floras. This may be because the two animal models are different, which possess different influences on the gut microbiota, and indirectly influence the effect of *H. erinaceus* polysaccharides on the dysbiosis of gut microbiota. Therefore, animal models are also an important consideration of the research on the applications of plant polysaccharides on gut microbiota.

Butyrate is one of the energy sources of colonocytes. It could modulate the gut barrier protection through inducing the consumption of O_2 in the epithelium [32]. In terms of metabolites, the significant ability of butyrate production of WHP attracted our attention because of the multiple beneficial effects of butyrate, such as decrease the pro-inflammatory cytokines; increase the learning and memory abilities in neurodegenerative diseases, and the abolishment of

lipopolysaccharide-induced depressive-like behaviors by the decrease in microglia activation [33]. The increase in butyrate production might be due to the fact that WHP contains a large amount of glucose, a main component of glucan which serves as a prebiotic and could be fermented by the gut microbiota leading to a supply for metabolism and utilization, then further promote the abundance of butyric acid-producing bacteria, such as the *Butyricicoccus*.

WHP is a glucose-rich polysaccharide. According to previous reports, the selective promotion of beneficial bacteria by glucose-rich polysaccharides is mechanistically attributed to their structural characteristics. Glucose-based polysaccharides containing specific glycosidic linkages, such as α-(1→4), α-(1→6), and β-(1→4), resist digestion by host enzymes but are hydrolyzed by bacterial glycoside hydrolases present in beneficial gut commensals including *Lactobacillus* and *Bifidobacterium* [34]. Zeng et al [34] demonstrated that gluco-oligosaccharides with lower degrees of polymerization (DP 2-3) are preferentially utilized by various *Lactobacillus* strains, with consumption rates exceeding 90%. Furthermore, Bai et al [35] reported that the prebiotic activity of glucose-rich polysaccharides is structure-dependent; polysaccharides containing T-Glcp, 1,3-Glcp, and 1,4-Glcp linkages are more readily fermented to produce SCFAs and promote beneficial bacterial proliferation. Consistent with these findings, Fu et al [36] showed that a glucose-predominant polysaccharide (53.93% glucose) significantly enriched SCFA-producing genera including *Clostridium_sensu_stricto*, *Gemmiger*, *Paraprevotella*, and *Bacteroides* during colonic fermentation. Collectively, these studies provide a mechanistic rationale for the selective prebiotic effects of glucose-rich polysaccharides.

The direct protective effects of *H. erinaceus* polysaccharides (HEP) on intestinal epithelial cells have been reported before. Using H_2O_2-induced IPEC-J2 cells, Li et al [37] demonstrated that HEP significantly scavenges reactive oxygen species (ROS), reduces apoptosis, and maintains mitochondrial membrane potential polarity through both mitochondrial and death receptor pathways. Similarly, Wang et al [38] showed that HEP (EP-1) protects Caco-2 cells from oxidative damage by increasing ROS scavenging efficiency and improving mitochondrial function, ultimately reducing epithelial cell apoptosis. Furthermore, a novel oligosaccharide from *H. erinaceus* was found to protect LPS-induced Caco-2 cells via the TLR4/NF-κB signaling pathway [39]. Notably, Wang et al [40] compared three HEP fractions and reported that the glucose-predominant polysaccharide (wHEP-1, glucose:galactose = 16.9:1.1) exhibited the strongest anti-inflammatory activity in LPS-induced Caco-2 cells, which is consistent with the monosaccharide composition of our WHP sample. Collectively, these studies provide strong evidence that HEP can directly act on enterocytes to exert protective effects against oxidative stress and inflammation, supporting the *in vivo* findings of the present study.

The Chinese herbal medicine, with the characteristics of natural and non-toxic, has a long history of application in China [41] though the mechanisms of some activities were not clear. The research on relationships between gut microbiota and herbal medicine provides a novel focus point for the development of Chinese herbal medicines. Some medicinal food is the first choice for daily health care. In China, the fruiting body of *H. erinaceus* often appears in the daily diet used as a delicacy or medicine for protecting the gastrointestinal tract thereby promoting the digestion of food. The protective effect on gastrointestinal tract is often closely connected to gut microbiota. From the results of this study, HEP could ameliorate the imbalance of the gut microbiota caused due to antibiotics, improve the level of metabolites, and thus alleviate AAD. However, there is still need for more experiments to confirm the potential molecular mechanism of the effects of WHP on AAD.

CONCLUSION

In conclusion, WHP showed beneficial effects on the rats with AAD induced by lincomycin hydrochloride through recovering the colon damage, improving the gut microbiota dysbiosis, and promoting the SCFAs level. WHP could adjust the relative abundance of *Lactobacillus*, *Butyricicoccus*, *Allobaculum*, and *Enterococcus* at the genus level and promoted the butyrate production. WHP might be used as a functional component to relieve the AAD depending on its ability to modulate the gut microbiota and homeostasis.

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