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Research Article

Houttuynia cordata Polysaccharide Ameliorates Chronic Inflammation-Induced Intestinal Impairment by Zonula Occludens-1 in Rats

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This study aimed to investigate the protective effects of *Houttuynia cordata* polysaccharide (HCP) against chronic intestinal inflammation in rats that were subjected to low-dose lipopolysaccharide once weekly for 6 weeks. Here, administration of HCP significantly restored morphological changes in the intestine along with enhancement of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase activities, and reduction of malondialdehyde contents. HCP treatment was also found to attenuate the inflammatory mediators nitric oxide, inducible nitric oxide synthase, total nitric oxide synthase, and interleukin-1beta (IL-lβ) and enhanced the production of short-chain fatty acids. Correspondingly, a significant elevation of zonula occludens-1 (ZO-1) was displayed in the intestine of HCP-treated rats, indicating that the intestinal mechanical barrier could be repaired by HCP treatment. Therefore, these findings suggested that HCP performed protective effects against chronic inflammation-induced intestinal impairment through alleviating inflammation, modifying the redox system, and recovering the intestinal mechanical barrier, mediated by the control of ZO-1 in rats.

1. Introduction

Overnutrition and/or imbalanced diets play a profound role in the immune response and often influence the inflammatory response, giving rise to chronic noncommunicable diseases (NCDs). Furthermore, there is growing evidence that chronic low-grade inflammation is a common denominator in most patients with NCDs, which is an important indicator of cardiovascular disease [1], cancer [2], chronic obstructive pulmonary disease [3], and digestive system diseases [4]. Recently, compelling evidence has been supported that chronic inflammation is detrimental to intestinal impairment, contributing to chronic noninfectious gastrointestinal diseases via the disruption of the integrity of the intestinal barrier and the reciprocal worsening effect of inflammation.

In the last few years, studies have proved that pectin can have a protective effect on intestinal health by directly affecting intestinal epithelial cells, acting as a substrate for the production of short-chain fatty acids (SCFAs), and regulating the intestinal flora [5, 6]. Pectic polysaccharides also possess an inhibitory property on intestinal inflammation through their macromolecular galacturonic acid main chain [7]. *Houttuynia cordata* polysaccharide (HCP), the main bioactive ingredient from *Houttuynia cordata*, has attracted increasing attention for its remarkable bioactivities such as immunomodulator [8], anti-inflammatory [9], antiviral [10], antioxidant [11], and anticancer [12]. Moreover, studies [8, 13] have confirmed that HCP was an acidic pectin polysaccharide containing a pyran ring structure with a monosaccharide composition of mainly galacturonic acid,

galactose, and glucose. Recently, the ameliorative effect of HCP on intestinal barrier damage caused by viral infection has been demonstrated [14]. Accordingly, we hypothesized that the onset and progression of intestinal dysfunction could be attenuated by HCP by recovering and maintaining the intestinal barrier in response to inflammatory insults.

In this work, lipopolysaccharide (LPS) was adopted to establish an intestinal inflammatory injury model, and dexamethasone (DEX) was employed as a positive control group to examine the therapeutic effectiveness among other groups in a bid to explore the possibility of HCP as an alternative agent for patients with digestive system diseases.

2. Materials and Methods

2.1. Materials and Reagents. *Houttuynia cordata* was purchased from Jiangxi Jiangzhong Chinese Medicine Decoction Pieces Co., Ltd. (Jiangxi, China). LPS (serotype O55: B5), and standards of acetic acid, propionic acid, n-butyric acid, isobutyric acid, n-valeric acid, and isovaleric acid were obtained from Sigma (St. Louis, Missouri, USA). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and nitric oxide synthase (NOS) kits were purchased from Nanjing Jiancheng Institute of Bioengineering (Jiangsu, China). Nitric oxide (NO) kit was purchased from Beyotime Biotechnology Company (Shanghai, China). IL-1β ELISA kit was purchased from Wuhan Boster Biotech Company (Wuhan, China). EDTA antigen repair solution and phosphate-buffered saline (PBS) were purchased from Google Biotechnology (Wuhan, China). Hydrochloric acid, ammonia, and hydrogen peroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used in this experiment were analytical grade provided by local chemical suppliers in China.

2.2. Extraction of Polysaccharide. According to our previous experimental method [15], HCP was isolated from the crushed *Houttuynia cordata* with 20 volumes of hot water at 95°C (2 h, twice). Furthermore, the two filtrates were pooled and the polysaccharide-rich fractions were precipitated by the final 80% volume fraction of ethanol. Subsequently, the obtained crude polysaccharide fraction was purified by deproteinizing 4 times via the Sevage method (polysaccharide solution : Sevage reagent = 4 : 1 v/v), dialyzed with tap water, distilled water, and ultrapure water for 24 h. After vacuum freeze-drying, the high purity of HCP was harvested. By using the high-performance gel permeation chromatography (HPGPC) method, the molecular weight of HCP was measured. HCP was dissolved in a 0.02 g/dL NaN_3 aqueous solution to 1.0 mg/mL and then determined by an Agilent 1260 high-performance liquid chromatography (HPLC) system (Agilent Technologies Corp., USA). Dextran standards with different molecular weights (2.0×10^6, 5.0×10^5, 7.0×10^4, 4.0×10^4, and 1.0×10^4 Da) were used to establish a standard curve. An Agilent 1260 Infinity system (Agilent Technologies, Amstelveen, The Netherlands) coupled with a refractive index detector (RID, G1362A) were used for the analysis. An Ultrahydrogel TM Linear Column (300 mm × 7.8 mm I.D., Waters Corporation, MA, USA) and an Ultrahydrogel Guard Column (40 mm × 6 mm I.D., Waters Corporation, MA, USA) were used for the experiments. The mobile phase was 0.02 g/dL NaN_3 (0.6 mL/min). The temperature of the columns and RI detector were maintained at 35°C. Structural information exhibited that HCP was an acid pectin polysaccharide with a pyranose structure, and its weight average relative molecular weight was about 3.87 × 105 Da. The polysaccharide is composed of eight monosaccharides: rhamnose, arabinose, galactose, glucose, xylose, mannose, galacturonic acid, and glucuronic acid. Scanning electron microscope analysis showed that the solid-state morphology of *Houttuynia cordata* polysaccharide was rod-shaped, silk-like, and spherical material intertwined with a loose structure.

2.3. Inflammation Model. Seventy male Sprague-Dawley (SD) rats (180–220 g) were supplied by Hunan Slack Jingda Laboratory Animal Co., Ltd. [Certificate Number SCXK (gan) 2018-0003]. Rats were maintained in a room at 25 ± 2°C with a relative humidity of 60 ± 5% (light-dark cycle 12 h/12 h) and given water ad libitum. The care and use of laboratory animals were conformed to animal standards by the Animal Care Review Committee (animal application approval number 0064257), Nanchang University, China, and strictly abide by animal experiment regulations referring to the United States National Institute of Health (NIH, Publication No. 85-23, 1996).

One week later, rats were randomly divided into 7 groups ($n = 10$ per group): the normal group (control), model group (LPS), dexamethasone positive control group (DEX), low-dose HCP group (HCPL), medium-dose HCP group (HCPM), high-dose HCP group (HCPH), and dexamethasone + low-dose HCP compound group (DEX.P). Except for the normal group, the rats in the other 6 groups were injected with LPS (0.4 mg/kg·BW) via the tail vein once a week for 6 weeks throughout the experiment. One hour after the initial injection of LPS, rats in the polysaccharide-treated group were gavaged with the corresponding dose of HCP solution (50, 100, 200 mg/kg·BW) [16], while rats in the positive control group and the compound group were intragastrically administrated with DEX solution (1 mg/kg·BW), DEX solution (1 mg/kg·BW) + low-dose HCP solution (50 mg/kg·BW), respectively. Meanwhile, rats in the normal group and model group were given intragastrically saline at corresponding volumes once a day for continuously 6 weeks. Finally, small intestinal tissues and colonic contents were collected and stored at −80°C.

2.4. Histopathological Observation of Small Intestine Slices. After washing with saline and absorbing excess water, the intestinal samples were fixed in a 10% neutral formalin solution for more than 48 hours and then treated with a gradient ethanol and benzene solution. Subsequently, the intestinal tissue was embedded in paraffin and prepared into approximately 3 μm-thick sections, followed by dewaxing, rehydration, and hematoxylin-eosin staining. Finally, the

structure and morphology of pathological sections of rat organs were observed by an optical microscope (Nikon, Tokyo, Japan) and photographed at 200× magnification.

2.5. Determination of ZO-1 Protein in Small Intestine Tissue. The expression of tight junction protein ZO-1 in small intestine tissue of chronic inflammation rats was determined by the immunohistochemical method. After the small intestine tissue sections were repaired with EDTA antigen repair buffer (pH = 9.0) and washed with PBS (pH = 7.4), the slices were incubated with a 3% H_2O_2 solution at room temperature away from light for 15 min. After PBS washing, slices were sealed in a wet box with a 10% goat serum working solution at room temperature for 15 min. Subsequently, the primary antibody was added to the tissue and incubated overnight in a refrigerator at 4°C. Tissue sections were incubated with secondary antibody at 37°C for 30 minutes, rewashed, and then fractionated by DAB staining, hematoxylin re-staining, 1% hydrochloric acid alcohol, and finally dehydrated and sealed. Finally, the positive staining was observed by an inverted light microscope (Nikon, Tokyo, Japan) and photographed under 400× magnification. Six visual fields were randomly selected, and Image-Pro Plus 6.0 software was used to analyze and measure the average IOD value of tissue protein expression, which was the ratio of measured integral optical density (IOD) to measured area.

2.6. Determination of Antioxidant Enzymes and MDA Contents. Part of the small intestine was weighed and made into a 10% homogenate with PBS. The supernatant was collected after centrifugation at 4°C for 10 min at a rotational speed of 3000 r/min. According to the instructions of the kit (SOD, CAT, GSH-Px, and MDA kits were obtained from Nanjing Jiancheng Institute of Bioengineering), the contents of SOD, CAT, GSH-Px, and MDA in the small intestine tissues of rats were determined.

2.7. Determination of NO, NOS, and IL-1β Contents. The contents of nitric oxide (NO), inducible nitric oxide synthase (iNOS), total nitric oxide synthase (tNOS), and interleukin-1 (IL-1) β in small intestine tissue were determined strictly according to the kit instructions (NO kit was obtained from Beyotime Biotechnology Company; NOS kits were purchased from Nanjing Jiancheng Institute of Bioengineering; IL-1β ELISA kit was purchased from Wuhan Boster Biotech Company).

2.8. Determination of pH and SCFAs in Colon Contents. 100 mg of colonic contents were loaded into sterile EP tubes, and ultrapure water was then added into the tubes at a ratio of 1 : 7 under low temperature. Subsequently, the sample was vortexed to homogenize, followed by an ice bath for 20 min. After sonication, homogenates were centrifuged at 4800 r/min for 20 min. Finally, the supernatant was separated for the determination of the pH value, and the SCFA content was determined using gas chromatography (Agilent Technologies, Santa Clara, CA).

2.9. Statistical Analysis. All experimental data were expressed as mean ± standard deviation (mean ± SD). The statistical significance of the experimental data was analyzed by SPSS 20.0 software and one-way ANOVA. Comparisons between groups were tested by the LSD method and $P < 0.05$ indicated the statistical significance.

3. Results

3.1. HCP Ameliorated Pathological Damage of the Small Intestine in Chronic Inflammatory Rats. As shown in Figure 1, intestinal mucosa epithelial cells in normal small intestine tissue were closely packed and neatly aligned, the intestinal villi were of normal length, the crypts were arranged regularly, and the lamina propria contained immune cells of normal quantity. Additionally, there were no signs of intestinal mucosal edema or congestion, which was the manifestation of inflammation in the model group. To be clear, a slight fracture of intestinal villi in the normal group might be due to mechanical damage to villi caused by excessive force during sampling. By comparison, the small intestinal epithelium in the model group was injured, the crypt was atrophied or ruptured, the mucous membrane was denuded and destroyed, and numerous intestinal villi were broken. Simultaneously, the inflammatory lesions in the small intestine were also obvious, including the increase of lymphocytes at the base of the crypt, the infiltration of inflammatory cells in the lamina propria of the mucosa, the significant dilation of blood vessels in the submucosa, and the exudation of inflammatory cells from the blood vessels. After HCP treatment, the benign histologic structure was achieved, which was expressed as follows: the structure of the intestinal epithelium was intact, the length of villi returned to normal, the crypts were arranged orderly, and only a minor portion of intestinal villi was ruptured. Meanwhile, the number of infiltrating inflammatory cells decreased significantly, and the swelling of the intestinal villi was alleviated. Similarly, the inflammatory injury of the small intestine in the DEX group and DEX.P group was reduced to varying degrees. The above pathological observation showed that HCP could improve the inflammatory injury in the small intestine of LPS-induced chronic inflammatory rats.

3.2. HCP Attenuated the Destruction of Tight Junction Proteins of the Small Intestine in Chronic Inflammatory Rats. ZO-1, an important tight junction protein, plays an irreplaceable role in the formation of intestinal barriers and the regulation of intestinal permeability [17]. As illustrated by immunofluorescence images of the control group (Figure 2(a)A), ZO-1 is expressed at the intercellular junction of intestinal epithelial cells, showing a uniform and continuous distribution of dark brown lines. In the model group (Figure 2(a)B), ZO-1 protein was scattered and could not be arranged into continuous lines, and its expression in some areas was weakened and showed yellowish, suggesting

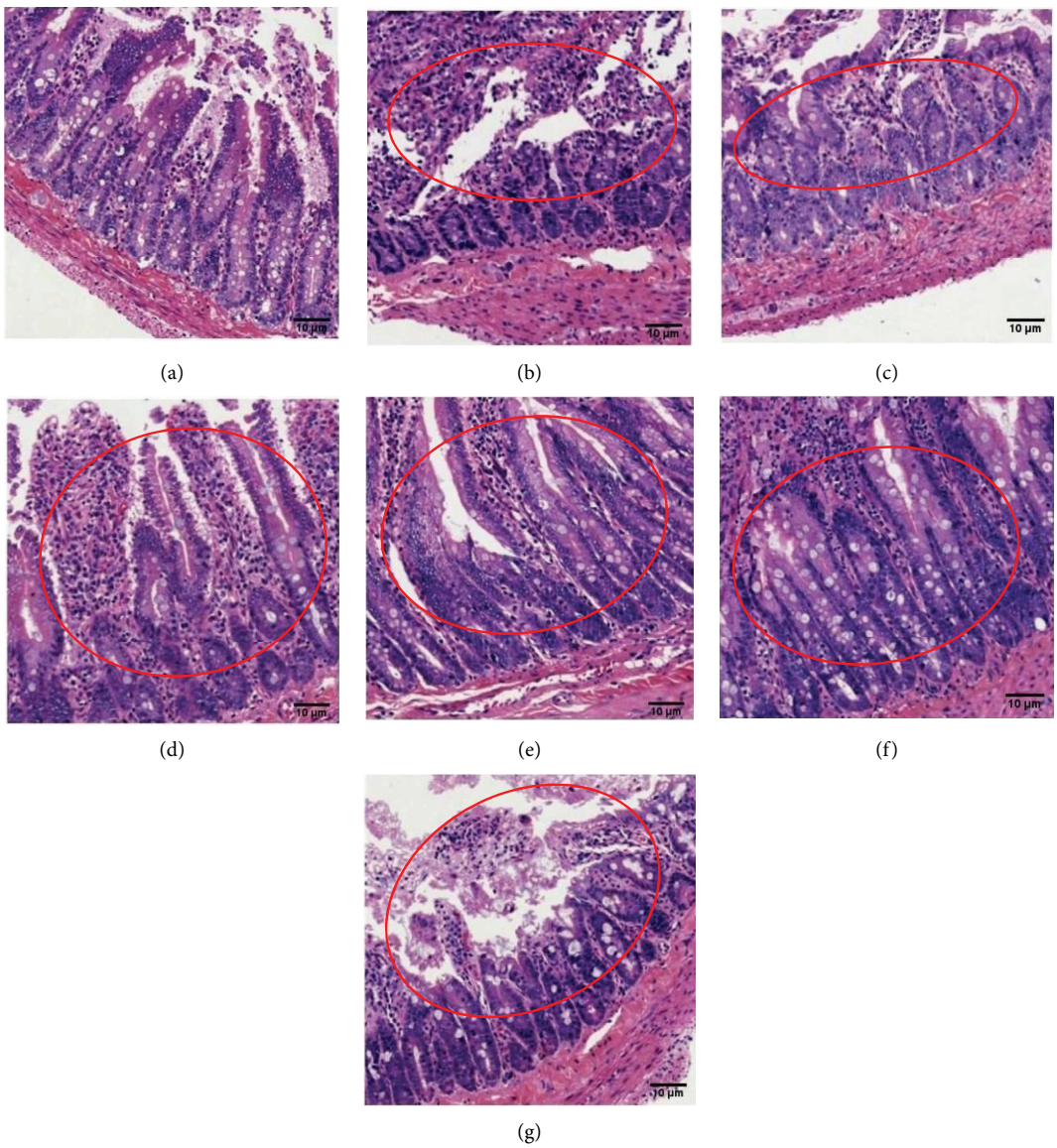
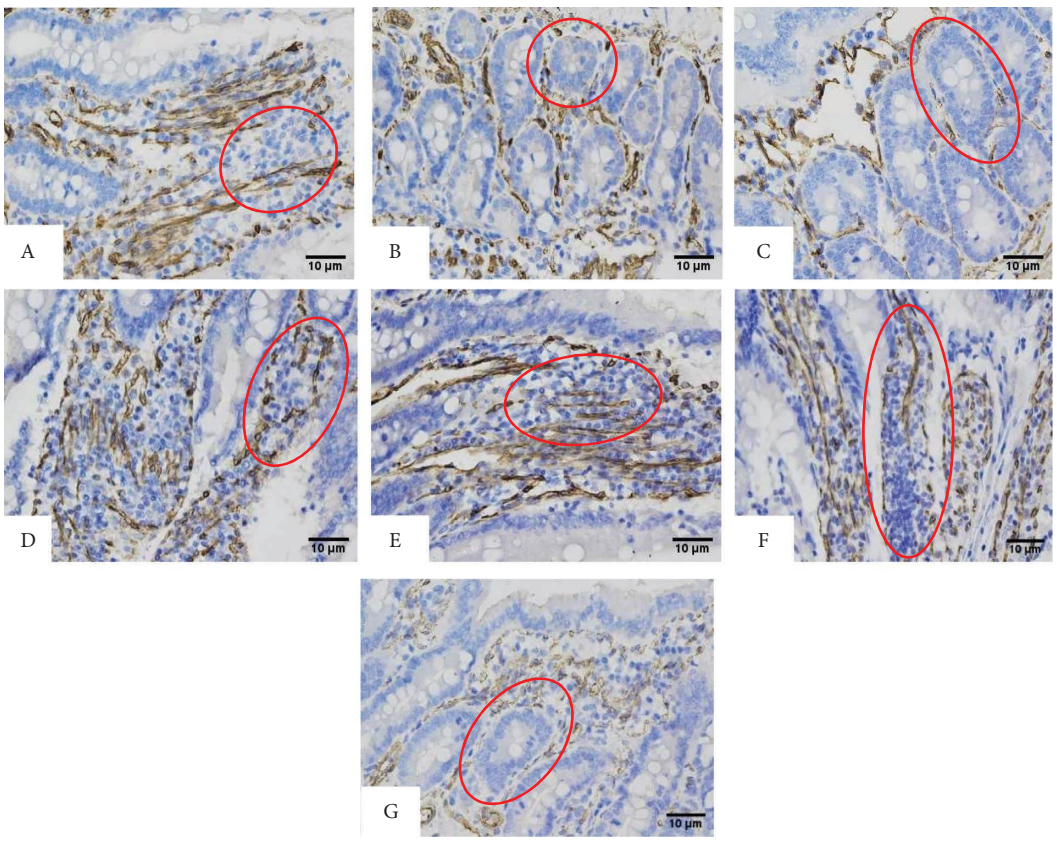


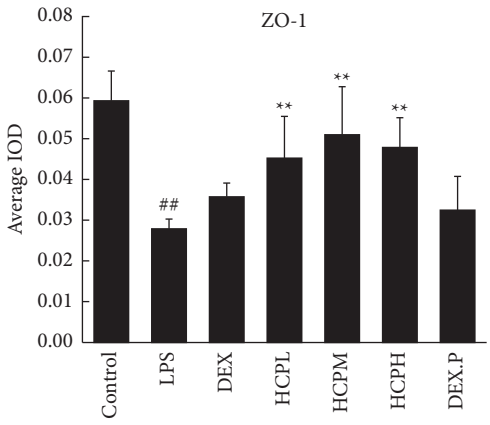
Figure 1: Observation on HCP treatment of LPS-induced pathological changes in the small intestine (×200). Except for the control group, the other 6 groups were infected with LPS. HCP treatment groups were intragastric with the corresponding dose of HCP solution (50, 100, and 200 mg/kg·BW), respectively. Meanwhile, the DEX group and DEX.P group were separately treated with DEX (1 mg/kg·BW) and DEX (1 mg/kg·BW) + low-dose HCP (50 mg/kg·BW). The inflammation and intestinal tissue damage were reflected by HE-staining intuitively. (a) Control group: morphology and structure of intestinal mucosa were normal and intact, villus arranged regularly; (b) LPS group: villus necrosed and exfoliated, inflammatory cells concentrated in the submucosa; (c) DEX group; (d) HCPL group; (e) HCPM group; (f) HCPH group; (g) DEXP group; (b–g): the swelling and fracture of intestinal villi ameliorated observably, infiltrating inflammatory cells reduced. Collectively, HCP treatment significantly relieved histological damage and inflammatory symptoms of the intestinal mucosa.

that the intestinal epithelial barrier was destroyed. In contrast, the expression of ZO-1 in the HCP treatment group was significantly enhanced, showing dark brown color and continuous lines (Figures 2(a)D~2(a)F). The corresponding quantitative chart (Figure 2(b)B) also showed the change in ZO-1 expression, as evidenced by the significant reduction of expression in the model group and varying degrees of enhancement in HCP-treated groups. Likewise, ZO-1 protein was expressed in DEX and DEX.P groups (Figures 2(a)C and 2(a)G), but it did not reach statistically significant differences. Therefore, the foregoing results indicated that HCP could promote the expression of ZO-1 protein in the small intestine of rats with chronic inflammation, which contributed to the integrity of the intestinal mucosal barrier.

3.3. HCP Repressed Oxidative Stress of the Small Intestine in Chronic Inflammatory Rats. As shown in Figure 3, compared with the normal group, the contents of SOD, CAT, and GSH-Px in the small intestine of the model group were significantly decreased, along with a significantly increased content of MDA. In contrast, the content of MDA in small intestine tissue in all HCP-treated groups decreased



(a)



(b)

Figure 2: Expression of ZO-1 in small intestinal tissues by immunohistochemical staining (×400). (a) Histological changes in the expression of ZO-1 in small intestinal tissues. (A) Control group; (B) LPS group; (C) DEX group; (D) HCPL group; (E) HCPM group; (F) HCPH group; (G) DEXP group: (A–G) Photomicrographs of ZO-1 immune-stained sections in the small intestine depicting integrity of the intestinal barrier; (b) quantitative analysis of immunohistochemical stains for ZO-1 in rats. Brown color (positive) stains for ZO-1 specific expression and light blue color (negative) denotes hematoxylin counterstain. Compared with the control group, the expression of ZO-1 protein in the LPS group was significantly reduced after injection of LPS in tail vein (once a week for 6 weeks) without any treatment, showing a scattered light-brown color. On the contrary, HCP treatment significantly enhanced the expression of ZO-1 protein as evidenced by a high-intensity brown color. $^{\#\#}$indicates extremely significant difference compared with the control group ($P < 0.01$), **indicates an extremely significant difference compared with the LPS group ($P < 0.01$).

significantly, and the contents of SOD, CAT, and GSH-Px in the high-dose HCP group increased markedly. Moreover, the contents of CAT exhibited a significant increase in the moderate-dose HCP and DEX groups, and the contents of SOD and CAT in the DEX.P group showed a dramatic increase in comparison with the model group. Altogether, the aforementioned results suggested that HCP could elevate the antioxidant capacity of small intestine tissue, suppress oxidative stress, and thereby exert a protective function on chronic intestinal inflammation.

3.4. HCP Downregulated Indices Associated with Intestinal Inflammation in Rats. As shown in Figure 4, compared with the control group, the amounts of NO, iNOS, tNOS, and IL-1β in the small intestine of the model group exhibited a significant increase. The contents of NO, iNOS, tNOS, and IL-1β in small intestinal tissues in all HCP-treated groups were decreased to varying degrees when compared with the model group. Among them, the low-dose HCP group revealed a significant decrease in the content of iNOS, tNOS, and IL-1β. Meanwhile, remarkably decreased contents of NO, iNOS, tNOS, and IL-1β were displayed in the remaining HCP treatment groups. Overall, these results revealed that the protective effect of HCP on the intestine correlates with its inhibition of inflammatory mediators and cytokine secretion in the small intestine of chronic inflammatory rats.

3.5. HCP Promoted SCFAs Production and Reduced the pH Value of the Colon in Chronic Inflammatory Rats. As shown in Figure 5, compared with the normal group, the contents of SCFAs including acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid, in the colon contents of rats in the model group were significantly decreased, accompanied by a significant increase in pH value, indicating that low-dose LPS stimulation in long-term treatment could reduce the secretion of SCFAs in rats. When compared with the model group, SCFA contents in all HCP-treated groups increased to varying degrees, and a concomitant decrease in pH value was also observed. Specifically, the contents of acetic acid, butyric acid, and valeric acid were significantly increased in the low-dose HCP group, and the pH value was remarkably decreased in the low-dose and medium-dose HCP groups. The experimental results implied that HCP could promote the secretion of SCFAs and thereby reduce the pH value in the intestinal tract of chronic inflammatory rats to a certain extent, which performed a protective role on intestinal health.

4. Discussion

A growing body of literature suggests that chronic inflammation plays an important role in chronic noncommunicable intestinal diseases such as inflammatory bowel disease and colorectal cancer [18, 19]. HCP was found to be an acid heteropolysaccharide made up of mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, xylose, galactose, and arabinose. Experiments revealed that HCP exhibited significant abilities to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, superoxide radical, and hydroxyl radical [11]. Further study proved that HCP suppressed chronic vascular inflammation induced by LPS in rats via calcium-sensing receptors and TLR4/NF-kB pathways [16]. The potential of HCP in the attenuation of inflammatory chaos was also being gradually explored [14, 20]. In this experiment, LPS was used to establish a model of intestinal inflammation, which has been shown to cause intestinal epithelial damage, leading to bacterial translocation, thereby causing systemic inflammatory responses [21]. It was also confirmed by our results that LPS infection caused intestinal histopathological damages, and HCP significantly alleviated LPS-induced intestinal epithelial injury, suggesting that the protective effect of HCP on the intestinal tract of rats was linked to the control of chronic inflammation.

The pathogenesis by which LPS-induced intestinal epithelial injury was multifactorial, but it was unquestionable that the intestinal mucosal barrier bore the brunt. The intestinal epithelial barrier was mainly composed of a mechanical barrier, an immune barrier, and a biological barrier, among which the main structure of the mechanical barrier was controlled by the tight junction between epithelial cells [22]. The destruction of the intestinal barrier promoted the penetration of pathogens in the intestinal lumen through the subepithelial tissues, causing mucosal and even systemic inflammatory reactions, and subsequently leading to a variety of intestinal diseases [23]. Disruption of tight junctions, excessive inflammatory responses, and oxidative stress all play pivotal roles in LPS-induced intestinal damage [24]. The tight junction protein ZO-1 was suggested to be one of the representative parameters to examine the status of intestinal barrier, and its reduction could lead to increased permeability [25]. Results consistent with previous studies [17], showed that LPS-induced inflammation was in association with the reduction of ZO-1 expression in rats. However, HCP could effectively reverse the downregulation of ZO-1, suggesting that HCP could maintain intestinal barrier integrity by regulating tight junction protein expression.

Disruption of the intestinal barrier increased intestinal permeability, which favored the entry of LPS into the intestinal mucosa and then in turn exacerbated tissue damage. Specifically, persistent LPS stimulus in chronic inflammation activated the immune system, followed by the recruitment of inflammatory cells to the site of the lesion, the release of excessive proinflammatory factors, and the disruption of immune homeostasis. During this period, reactive oxygen species (ROS) were generated, which amplified the inflammatory effects. Generally, ROS released in the vicious cycle of inflammation and oxidative stress caused obvious damage to tissues and organs, leading to the onset and development of chronic diseases [26]. Therefore, several inflammatory and oxidative stress indicators were investigated. Results showed that HCP treatment reduced the levels of inflammatory mediators NO, iNOS, tNOS, and proinflammatory factor IL-1β. Elevation of the antioxidant enzymes SOD, CAT, and GSH-Px and attenuation of MDA contents were also shown during HCP-mediated anti-inflammatory responses in rats subjected to LPS.

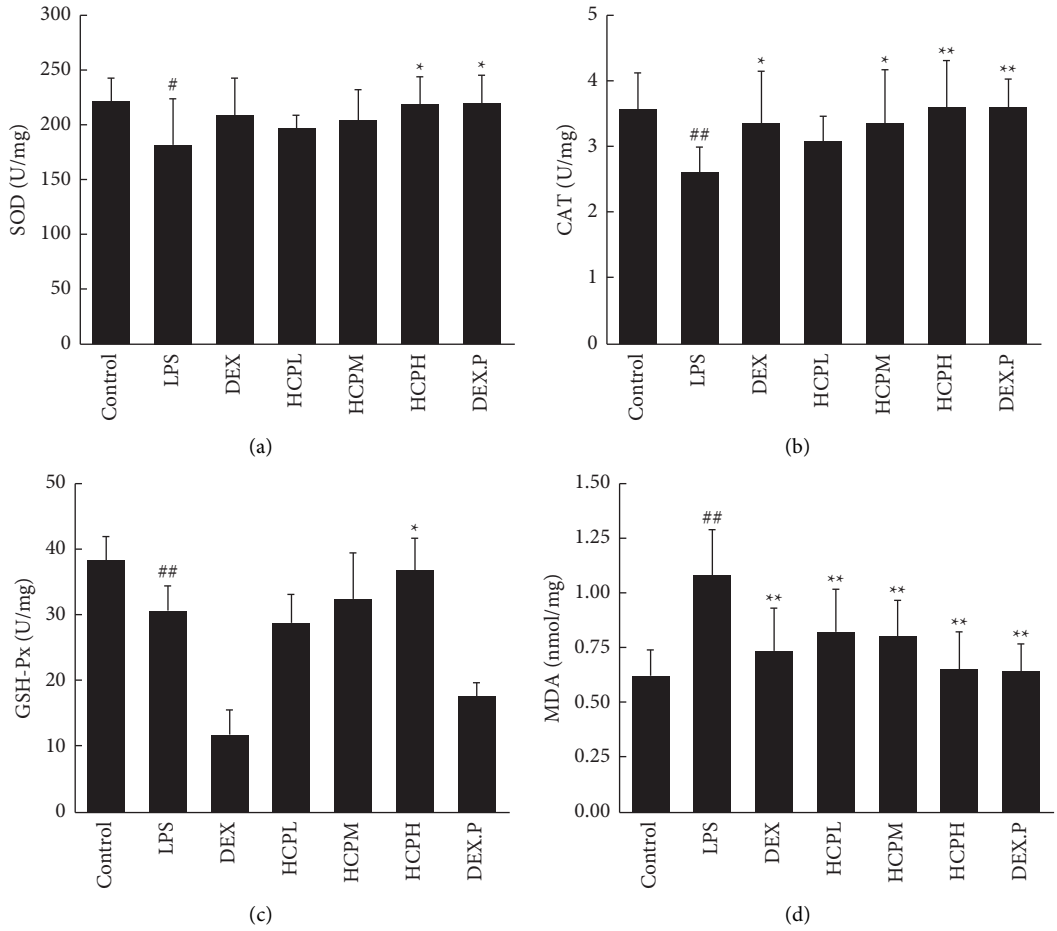


Figure 3: Effect of HCP on indices related to oxidative stress in the small intestine. (a) Effect of HCP on SOD secretion in small intestinal. (b) Effect of HCP on CAT secretion in small intestinal. (c) Effect of HCP on GSH-Px secretion in small intestinal. (d) Effect of HCP on MDA secretion in small intestinal. A chronic inflammatory response caused by LPS stimulation for 6 weeks leads to the accumulation of MDA concurrent with the reduction of antioxidant substances including SOD, CAT, and GSH-Px. Therapy with HCP markedly blunted LPS-induced oxidative stress in the small intestine. $^{\#}$indicates significant difference compared with control group ($P < 0.05$), $^{\#\#}$indicates extremely significant difference compared with control group ($P < 0.01$); *indicates significant difference compared with LPS group ($P < 0.05$), **indicates extremely significant difference compared with LPS group ($P < 0.01$).

Concretely, IL-1β was the upstream initiator of the inflammatory cascade [27]. Meanwhile, NO was a free radical catalyzed by the nitric oxide synthase (NOS) family. eNOS, nNOS, and iNOS were the three common isozymes of the NOS family, and their total content was indicated by tNOS [28]. Activation of NOS during cellular stimulation by inflammation promotes NO synthesis, which subsequently exacerbated oxidative stress and ultimately led to tissue damage. MDA, a harmful product formed by excessive ROS, damaged the permeability of the cell membrane [29]. It has been demonstrated that SOD, CAT, and GSH-Px, intracellular antioxidant active substances, could effectively scavenge a considerable number of ROS generation in inflammatory responses [30]. Therefore, the above results indicated that the protective roles of HCP against chronic inflammation-induced intestinal barrier impairment could be attributed to the inhibition of proinflammatory mediators and cytokines and the modification of the redox system in intestinal tissue.

Pectic polysaccharides have been shown to repair and strengthen the intestinal barrier and reduce inflammation by promoting the synthesis of SCFAs [6, 7]. SCFAs, well-known as volatile fatty acids, were referred to organic fatty acids that were transferred from polysaccharides, cellulose, pectin, and resistant starch that pass undigested through the small intestine and reach the colon, where they were mainly fermented by intestinal flora, among which acetic acid, propionic acid, and butyric acid account for about 95% of the total content of SCFAs [31]. Furthermore, SCFAs could strengthen the intestinal mucosal barrier, attenuate inflammation, modulate immunity, and exert multiple protective effects on the body [32, 33]. Several investigations [34] also found that SCFA could serve an anti-inflammatory function by lowering the pH value of the intestinal lumen. The changes in inflammatory mediators, oxidation indices, ZO-1 protein, and SCFA content measured in the present study also confirmed the role of SCFA mentioned above. Furthermore, our results showed that the colonic SCFAs

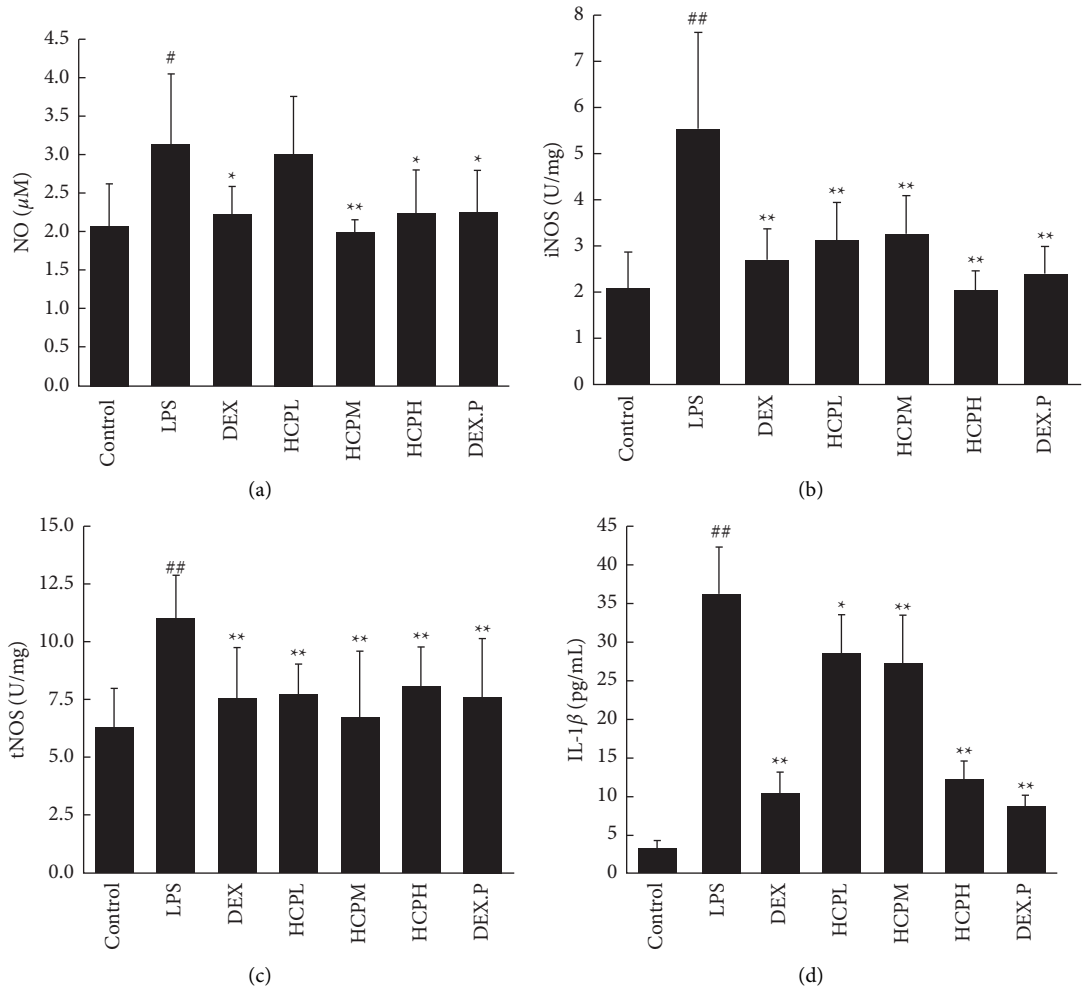


Figure 4: Effects of HCP on inflammation-associated cytokines and mediators in the small intestine. (a) Effect of HCP on NO secretion in small intestinal. (b) Effect of HCP on iNOS secretion in small intestinal. (c) Effect of HCP on tNOS secretion in small intestinal. (d) Effect of HCP on IL-1β secretion in small intestinal. Inflammatory mediators NO, iNOS, tNOS, and proinflammatory factor IL-1β were involved in the LPS-induced inflammatory cascade in the small intestinal, corresponding to the increase of related indexes in the LPS group. The therapeutic effect of HCP presented as decreased contents of NO, iNOS, tNOS, and IL-1β in small intestinal. $^{\#}$indicates significant difference compared with control group ($P < 0.05$), $^{\#\#}$indicates extremely significant difference compared with control group ($P < 0.01$); *indicates significant difference compared with LPS group ($P < 0.05$), **indicates extremely significant difference compared with LPS group ($P < 0.01$).

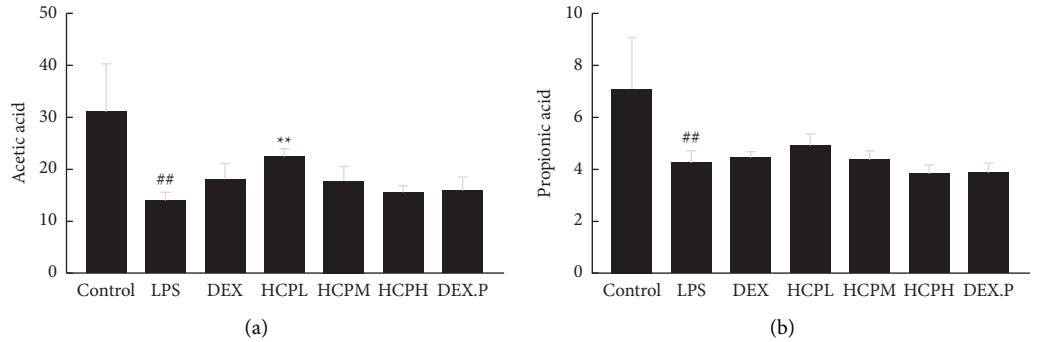


Figure 5: Continued.

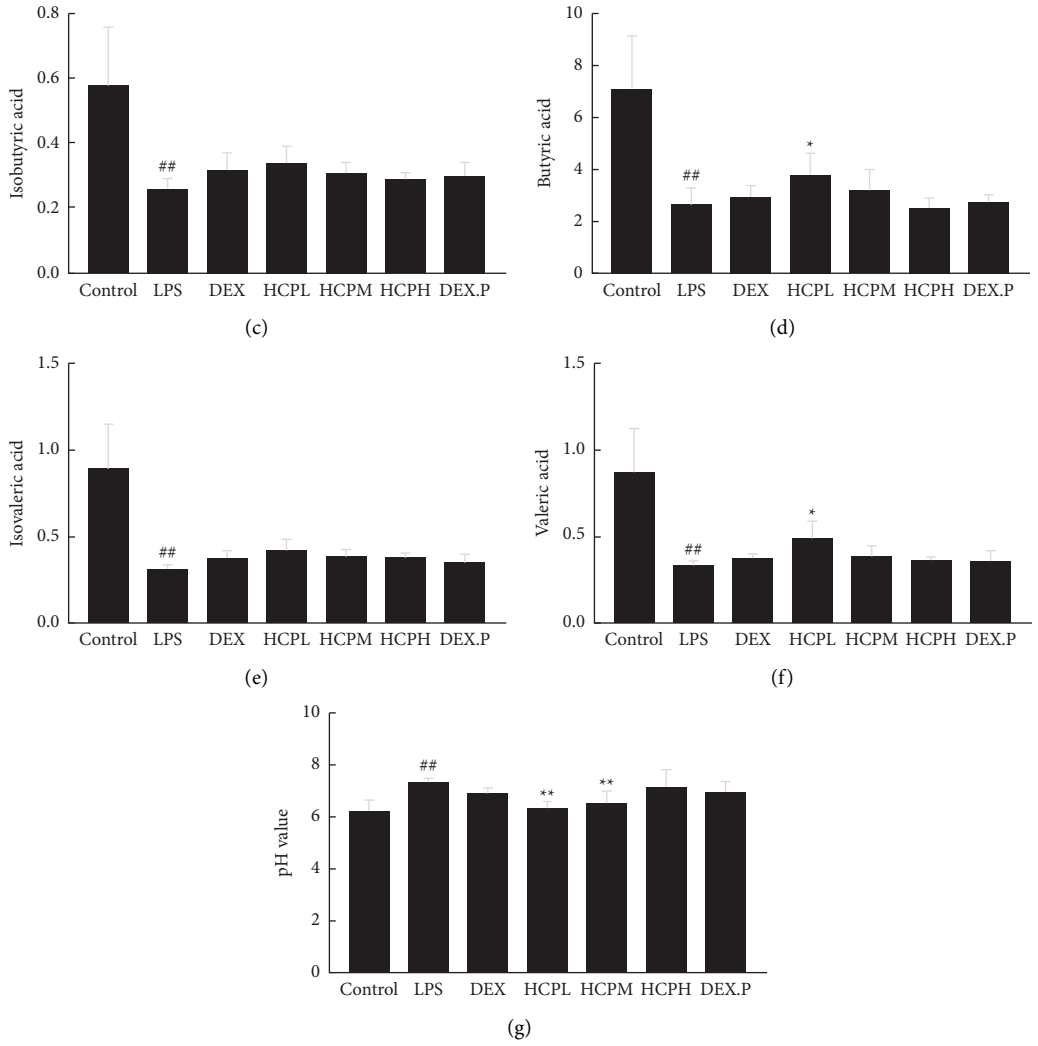


Figure 5: Effects of HCP on SCFAs and pH value in colonic contents of chronic inflammatory rats. (a–f) The effect of HCP on the content of SCFAs, which includes: (a) acetic acid; (b) propionic acid; (c) isobutyric acid; (d) butyric acid; (e) isovaleric acid; (f) valeric acid. (g) Effect of HCP on pH value of intestinal contents. Long-term LPS stimulation significantly diminished SCFAs secretion in colonic contents and interfered with intestinal acid-base homeostasis of chronic inflammatory rats in the LPS group. HCP suppressed inflammation by regulating anti-inflammatory compounds SCFAs, as evidenced by the enhancement of SCFAs content and the lower pH value in HCP treatment groups when compared to the LPS group. $^{\#\#}$indicates extremely significant difference compared with control group ($P < 0.01$); *indicates significant difference compared with LPS group ($P < 0.05$), **indicates extremely significant difference compared with LPS group ($P < 0.01$).

contents in the model group decreased significantly, and the pH value increased markedly. Compared with the model group, low-dose HCP could significantly increase the contents of SCFAs, such as acetic acid, n-butyric acid, and n-valeric acid, and reduce the pH value, indicating that HCP had an enhancement effect on the improvement of intestinal epithelial integrity and restored secretion of SCFAs in chronic inflammatory rats. Overall, in this study, we verified the anti-inflammatory and antioxidant effects of HCP on LPS-induced chronic inflammation in rats. We then further demonstrated that HCP could repair the damaged intestinal barrier by promoting the expression of ZO-1 protein as well as the production of SCFAs. However, the effect of HCP on intestinal flora in rats with chronic inflammation has not been studied in this study.

5. Conclusions

The present study investigated the effects of HCP on intestinal injury, inflammation, oxidative homeostasis, and metabolic phenotype in LPS-induced chronic inflammation rats. The results showed that HCP rebuilt the intestinal pathological structure and strengthened the repair of the intestinal barrier composed of the tight junction protein ZO-1. In addition, HCP improved the antioxidant capacities of small intestinal tissue and downregulated the levels of inflammatory mediators and proinflammatory factors. At the metabolic level, HCP increased SCFAs content in the colon, conferring a probiotic role. Therefore, this study revealed the significant protective effect of HCP on intestinal injury and provided new insights into the future treatment of chronic

inflammatory intestinal injury. However, the specific effects and mechanisms of HCP on intestinal damage remain to be further elucidated. Due to its ability to inhibit chronic intestinal inflammation, HCP might inhibit colon cancer. In the later stage, we can combine metabonomics, microbiology, and other research methods to find biomarkers. Further studies on the correlation between the changes of body metabolites and intestinal flora should also be carried out so as to explore the beneficial effects of HCP on chronic inflammation and colon cancer from multiple perspectives.

Data Availability

The authors declare that datasets are available on request.

Additional Points

Practical applications. In recent years, chronic noncommunicable diseases have gradually become a global challenge. This study verified the protective effect of HCP, a common active substance of *Houttuynia cordata*, against chronic inflammation of the intestinal tract. Experimental results showed that HCP mitigated intestinal disorders via the modification of the redox system and inflammation, and repaired intestinal mechanical barrier damage by upregulating ZO-1 expression and promoting SCFAs production. Therefore, this study provides a basis for the prevention of chronic noncommunicable diseases by HCP and helps to develop the clinical application of *Houttuynia cordata*.

Disclosure

Ye-Hao Song and Hui-Ting You are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wen-Juan Li conceptualized the study, performed formal analysis, provided funding acquisition, performed project administration, provided resources, performed supervision, wrote, reviewed, and edited the manuscript. Ye-Hao Song conceptualized the study, performed project administration, performed data curation, performed investigation, performed the methodology, provided software, performed validation, and contributed to visualization. Hui-Ting You conceptualized the study, performed data curation, performed investigation, provided software, contributed to visualization, and wrote the original draft. Ting Sang provided funding acquisition. Yu-Fei Yao conceptualized the study, provided funding acquisition, performed project administration, performed investigation, and performed the methodology. Su-Mei Chen performed data curation. Min Wan contributed to visualization. Ye-Hao Song and Hui-Ting You contributed equally to this work.

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