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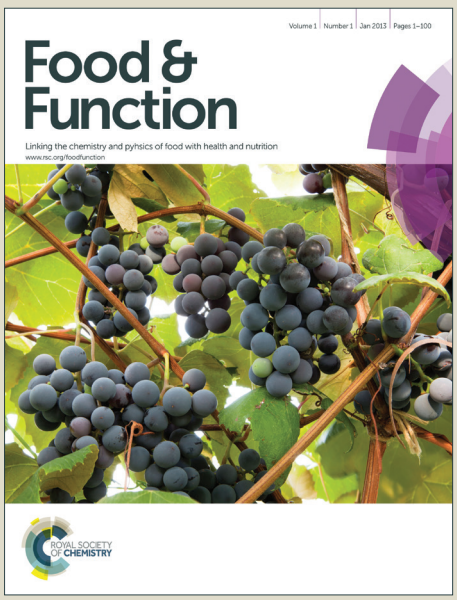

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Wild jujube polysaccharides protect against experimental inflammatory bowel disease by enhanced intestinal barrier function

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Abstract:

Dietary polysaccharides provide various beneficial effects for our health. We investigated the protective effects of wild jujube (*Ziziphusjujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou*) sarcocarp polysaccharides (WJPs) against experimental inflammatory bowel disease (IBD) by enhanced intestinal barrier function. Colitis was induced in rats by intrarectal administration of TNBS. We found that WJPs markedly ameliorated the colitis severity, including less weight loss, decreased disease activity index scores, improved mucosal damage in colitis rats. Moreover, WJPs suppressed the inflammatory response via attenuation of TNF-α, IL-1β, IL-6 and MPO activity in colitis rats. And then, to determine the effect of WJPs on intestinal barrier, we measured the effect of WJPs on the transepithelial electrical resistance (TER) and FITC-conjugated dextran permeability in Caco-2 cells stimulation with TNF-α. We further demonstrated that the alleviation of WJPs to colon injury was associated with barrier function by assembly of tight junction proteins. Moreover, effect of WJPs on TER was abolished by specific inhibitor of AMPK. AMPK activity was also up-regulated by WJPs in Caco-2 cells stimulation with TNF-α and in colitis rats. This study demonstrates that WJPs protect against IBD by enhanced intestinal barrier function involving the activation of AMPK.

Key words: *Ziziphusjujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou,* polysaccharides, inflammatory bowel disease, intestinal barrier, tight junction, AMPK

1. Introduction

The intestinal epithelial cells line the luminal surface of the intestinal mucosa and provide a physical barrier to the diffusion of pathogens, toxins, and allergens from the lumen into the circulatory system [1]. Disruption leads to increased translocation of these noxious, which can be important triggers for chronic activation of intestinal immune system [2]. The defects in the barrier function play a crucial role in the pathogenesis of inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis [3, 4]. Despite advances in treatment options for IBD, still a great number patient cannot achieve remission. Current pharmacological therapies for IBD long-term management rely on anti-inflammatory drugs, which can result in serious side effects, including secondary infections and immunosuppression [5], highlighting the need for novel therapeutic targets. The promotion and protection of intestinal barrier integrity may be therapeutic approaches for IBD [6].

The pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α) is a critical mediator in the inflammatory process in IBD. Moreover, TNF-α may be a pathophysiologically relevant regulator of intestinal epithelial tight junction permeability [7, 8]. It was confirmed that TNF-induced barrier loss was not only due to epithelial apoptosis [9, 10]. Another major component of TNF-induced increases in paracellular permeability is enhanced flux across the nonrestrictive class of pores, which is related to tight junctions (TJ) [10, 11]. The formation of tight junctions (TJ) between epithelial cells is one major component of the intestinal barrier. The TJ complexes consist of transmembrane and cytosolic proteins. A wide spectrum of proteins and 3 integral transmembrane proteins, occludin, claudins, and junctional adhesion molecule (JAM), have been identified to date[12]. These interact with cytosolic plaque proteins, including zonula occludens (ZO) proteins, symplekin, and cinglin [13], which in turn anchor the TJ complexes to perijunctional actin cytoskeletal rings within the cells [2]. Previous studies have shown that the association of TJ proteins with the actin cytoskeletal ring is vital for understanding the function and status of the intestinal barrier [14].

A considerable body of evidence indicates that the regulation of tight junction assembly involves activation of the AMP-activated protein kinase (AMPK) [15]. AMPK, a serine/threonine kinase, functions as a cellular fuel gauge to maintain energy balance at the cellular level [16]. AMPK regulates metabolic pathways in glucose and fatty acid metabolism and protein synthesis [17]. The binding of AMP to AMPK allows it to be phosphorylated on Thr-172, resulting in its activation [18, 19]. The tight junction assembly is impaired when AMPK activity is down-regulated by a genetic manipulation strategy [15, 20, 21]. The assembly of tight junctions is facilitated by the up-regulation of AMPK activity and is inhibited by the down-regulation of AMPK activity [20].

Wild jujube (*Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F.Cho*u) is a thorny, rhamnaceous deciduous plant widely distributed in northern China [22]. The fruits of wild jujube are much admired for their high nutritional value. However, the studies mainly focused on its seed, which is a famous Chinese medicine (known as suanzaoren in China). The sarcocarp of wild jujube which are rich in polysaccharides [23], have an exquisite taste and attractive red colour. There has been reported some polysaccharides can improve hepatic injury, experimental colitis, obese and so on [24-26]. Previously we evaluated the chemical composition of the crude polysaccharides from wild jujube sarcocarp and found the crude polysaccharides exert antioxidant activity and hepatoprotective effect [24]. However, the protective effects of wild jujube sarcocarp polysaccharides (WJPs) against IBD and its underlying mechanism have yet to be elucidated.

The aim of the present study was to test the hypothesis that the effect wild jujube sarcocarp polysaccharide (WJPs) on the intestinal barrier is mediated by the regulation of the assembly of tight junctions involving the activation of the AMPK. Additionally, we designed an *in vivo* study to assess the therapeutic potential of polysaccharide in 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colitis, which resembles Crohn's disease in humans.

2. Materials and methods

2.1 Preparation WJPs

The polysaccharide from wild jujube was extracted as previously described [24]. The contents of polysaccharides in WJPs were determined to be 80.4% and it contained relatively high contents of uronic acid (15.3%). The polyphenols, flavonoids, total anthocyanins, total saponins and total alkaloids were found to be absent in WJPs. Furthermore, HPLC analysis showed that WJPs is an acidic heteropolysaccharide, rich in arabinose glucose (38.59%), arabinose (23.16%), galacturonic acid (17.64%) and galactose (10.44%).

2.1 Reagents and cell culture

The Caco-2 cells and RAW264.7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco by Invitrogen, CA, USA), containing 10% fetal bovine serum (FBS, Gibco). All of the media contained 100 IU/ml penicillin and 100 μg/ml streptomycin. The cells were grown in 25-cm^2 flasks at 37 ℃ in a 5% CO_2-humidified incubator. The RAW264.7 cells were seeded onto the 96-well plate. LPS (100 ng/ml)-stimulated RAW264.7 were incubated without or with WJPs. The Caco-2 cells were seeded into permeable polyester membrane filter supports (Transwell, 12 mm diameter, 0.4 μ m pore size; Corning Costar Co., Cambridge, MA, USA) at a density of 0.5×10^6 cells/cm^2. All experiments were conducted on days 10–11 post-seeding when the cell monolay ers reached a plateau of the transepithelial electrical resistance around 1000–1200 Ω·cm^2. The medium was refreshed every 3 days.

2.2 Measurement of intestinal barrier function

Intestinal barrier function was evaluated by measurement of transepithelial electrical resistance (TER) and unidirectional flux of FITC-conjugated dextran (FD-4; average molecular weight 4000 Da, Sigma) in Caco-2 cell monolayers in Transwell filter supports [12, 27]. The cell monolayers show the TER of 1000–1200 Ω·cm^2 on days 10–11 post-seeding using a Millicell-ERS system (Millipore, Bedford, MA, USA), high alkaline phosphatase and sucrase activities (data not shown). Caco-2 cells were

stimulated with TNFα (20ng/mL) for 24 h. WJPs (200, 100, 50, 25 and 12.5 μg/ml) was administered to the apical wells at the same time, and the cells were incubated for 24 h. FD-4 (100 μM) was injected into the apical wells at 21 h after WJPs administered, and the flux into the basal wells was assessed for 3 h. FD-4 concentrations in the basal solutions were measured using a fluorescence plate reader (excitation: 495 nm, emission: 520 nm).

2.3. Experimental animals

Adult male Sprague-Dawley rats (weighing 200-250g, Wuhan University Laboratory Animal Center) were housed on a 12h light–dark cycle at 25 ± 2℃, and in a relative humidity of 60–80%. Animals were fed on a diet of standard pellets and water. The animals were allowed to acclimate to the housing conditions for 5 days prior to experimentation. Animal study followed ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines and was approved by The Institutional Animal Care and Use Committee (IACUC), Wuhan University Center for Animal Experiment, Wuhan, China.

2.4. Animal model of colitis

Colitis was induced in the rats by rectal administration of Trinitrobenzene sulfonic acid (TNBS) (Sigma) into the colons in a dose of 100 mg/kg, dissolved in 50% solution of ethanol as described by others [28]. Briefly, 2 ml/kg of TNBS－ethanol solution (50 mg/ml) was administered into the colon at the depth of 8 cm from the rectum with the use of a soft polyethylene catheter following a 12 h fast. The rats were positioned in the Trendelenburg position for one minute in order to avoid loss of TNBS solution *via* the rectum. Normal control animals received rectal administration of 50% ethanol solution at 2 mL/kg without TNBS during induction.

2.5. Experimental protocol and sample preparation

The animals were treated daily, starting 3 days after colitis induction. Animals in the normal control group (Normal control) received ethanol vehicle with no TNBS during induction and received oral saline during treatment. TNBS-induced colitis animals were randomly divided into 4 treatment groups: the rats in test groups received oral doses of 20, 40 and 80 mg/kg bw.day of WJPs (0.1 mL/10g, ig),

respectively. Clinical symptoms, including the amount of food consumed, consistency and frequency of stools and the change of body weight were monitored until tissue harvest.

2.6. Evaluation of colitis progression.

Body weights were recorded daily. Disease activity index (DAI) was based on weight loss, stool consistency and blood in stools [29], which is expressed as the equation: DAI = (body mass loss + fecal viscosity + rectal bleeding)/3. Briefly, score was assigned for each item to calculate DAI as follows: (i) Percentage of weight loss: 0, none; 1, 1–5%; 2, 6–10%; 3, 11–15%; 4, >15%. (ii) Stool consistency: 0, normal; 2, loose stool; 4, diarrhea. (iii) Blood in stools: 0, hemoccult (-); 1, hemoccult (±); 2, hemoccult (+); 3, hemoccult (++); 4, gross bleeding.

Rats were killed on day 14 with colons removed. Colons were measured and cut into sections. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, slightly cleaned in physiological saline to remove faecal residues and weighed. Macroscopic damage was quantified by the pathologists who were blinded to the group, as previously described by others [30]. Briefly, scoring of macroscopic colon damage was as follows: 0, no colonic damage; 1, hyperaemia and no ulcer; 2, linear ulcer and no colonic wall thickening; 3, linear ulcer and colonic wall thickening in one area; 4, colonic ulcer at multiple areas; and 5, major ulcer and perforation.

2.7. Measurement of colonic mucosal permeability

Colonic mucosal permeability was assayed using a modification of the method described by Lange et al[31]. Briefly, a small catheter was placed into the distal rectum via the anus under general anaesthesia, and then the proximal colon and distal rectum were ligated. 0.5 ml of 1.5% (w/v) Evan's blue (Sigma) in PBS was instilled in the closed loop of the colon. After 120 min of exposure to Evan's blue, the rats were sacrificed and the colon was removed. The dissected colon was opened and rinsed three times in 6 mM acetylcysteine to remove any unabsorbed Evan's blue. The colon

was then incubated with 2 ml of formamide at 50 ℃ for 48 h to elute the Evan's blue. The measurements were performed in a spectrophotometer at a wavelength of 660 nm.

2.8. Histological examination

Histopathological studies were performed on paraffin embedded, 4 μm thick distal colon sections, stained with haematoxylin and eosin. The colonic pathological changes were observed and evaluated by two trained independent researchers using a modified histopathological score formula[26]: (i) infiltration of acute inflammatory cells: 0 none, 1 mild, 2 severe; (ii) infiltration of chronic inflammatory cells: 0 none, 1 mild, 2 severe; (iii) fibrin deposition: 0 negative, 1 positive; (iv) submucosal oedema: 0 none, 1 focal, 2 diffuse; (v) necrosis of epithelial cells: 0 none, 1 focal, 2 diffuse; and (vi) mucosal ulcer: 0 negative, 1 positive.

2.9. Measurement of cytokine levels and leukocyte invasion in serum and colonic mucosa.

After collection, peripheral blood was centrifuged for 5 min at 1500 g, and serum was collected. To quantify colonic tissue cytokines, 50 mg of colonic tissue was extracted using 500 μl of 5 M guanidine HCl and 50 mM Tris–HCl (pH 8.0) with a protease inhibitor (Beyotime, Shanghai, China). Levels of TNFα, IL-1β and IL-6 in serum and colonic mucosa were measured by ELISA kits (KYM, Beijing, China) according to the manufacture's recommendations. Besides, MPO activity, an index of leukocyte recruitment, was measured with an MPO assay kit obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China) according to the instruction manuals.

2.10. Western blotting

Equal amounts of protein were subjected to Western blot analysis as previously described [32]. Protein lysates from both rats and Caco-2 cells were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The blocked membranes were subsequently incubated with antibodies specific against occludin, claudin-1, claudin-4, and ZO-1, and β-actin (Santa Cruz Biotechnology). Antibodies specific against AMPK as well as those specific for their respective

phosphorylated forms, p-AMPK were obtained from Cell Signaling Technology (Beverly, MA, USA). Immunoreactive bands were detected by incubating the membranes with anti-rabbit, anti-goat, or anti-mouse IgG antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and using chemiluminescence reagents to quantify the relative expression (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA).

2.11. Statistical analysis

The data were expressed as the means ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student's t-test with a Bonferroni correction. $P<0.05$ was considered to be statistically significant.

3. Results

3.1 WJPs attenuated TNBS-induced experimental colitis

Treatment with WJPs resulted in prominent protection from colitis as assessed by body weight, disease activity index (DAI) scores, colon length, wet weight (colon weight /cm) (Fig. 1) and histopathological damage of the colon (Fig. 2). The main outward signs of TNBS-induced colitis were severe diarrhea and loss of body weight. While the model group rats (TNBS treated only) gradually lost weight, WJPs significantly (p<0.05) attenuated body weight loss associated with TNBS administration (Fig. 1A). WJPs significantly improved DAI scores, colon length and wet weight in TNBS colitis (Figures 1B, C, D). These data were confirmed by the macroscopic examination of colon. Macroscopic examination of the colon after TNBS induction showed hyperemia, thickening of the bowel, and a large area of ulceration (Figures 1E). Interestingly, WJPs mitigated these changes and diminished the severity of colonic injury as compared to TNBS colitis group.

Histological examination (Fig. 2) of the colon tissue from the model group revealed significant tissue injury with high scores of microscopic damage indicating focal necrosis of mucosa and submucosal and ulceration of the colonic mucosa with loss of lining epithelium. In contrast, WJPs significantly ameliorated the signs of colitis and revealed an intact architecture of colon tissues. The comparison of histological scores also showed a significant relief of colon inflammation and preservation of colon cytoarchitecture in WJPs-treated group, compared with the model group. These findings suggest the beginning of re-epithelization and healing in the WJPs -treated rats.

In most experimental models of chronic intestinal inflammation, including TNBS-induced colitis, various inflammatory cytokines were shown to be overexpressed in the intestinal tissue [33]. In the present study, the expression of TNF-α, IL-1β and IL-6 both in serum and colonic tissues were measured using ELISA kits shown in Fig. 3. The expression of TNF-α, IL-1β and IL-6 both in serum and colonic tissues were notably increased following TNBS challenge. Administration of WJPs to rats significantly inhibited the elevated expression of these cytokines after TNBS

challenge. Besides, the leukocyte invasion was monitored by assessing myeloperoxidase (MPO) activity in colonic tissues. Leukocyte invasion to colonic tissues was confirmed by a 3-fold increase of MPO activity, as compared to the control group. WJPs administration afforded a significant reduction of MPO activity as compared to TNBS group. These findings suggest that WJPs could be a potent therapeutic agent for the treatment of patients with IBD.

3.2 WJPs enhances the barrier function in Caco-2 cells

TNF, a critical mediator of Crohn's disease, has been recognized as a regulator of tight junction permeability [34, 35]. Thus, we tested whether WJPs can reduce the inflammatory cytokines release. However, there is no effect of WJPs on the TNF-α release from RAW264.7 cells (Fig. 4 A). And then, we investigated whether WJPs can protect the cells against TNF-α-induced barrier dysfunction. The stimulation with TNF-α (20 ng/ml) in Caco-2 cell resulted in a decrease in TER (Fig. 4 B) and an increase in FD-4 permeability (Fig. 4C). WJPs (200, 100, 50 and 25 μg/mL) significantly reduced TNFα-induced changes in TER and FD-4 flux. Moreover, WJPs treatment at the dose of 100 μg/mL exerts the greatest protective effect on the barrier function in Caco-2 cell. WJPs were administered at this dosage in the following *in vitro* studies.

3.3 AMPK activation is involved in the WJPs regulation of the barrier function in Caco-2 cells

AMPK has been shown to modulate the TJ integrity in Caco-2 Cell Monolayers [36]. To determine whether AMPK is involved in the pathway through which WJPs regulates the barrier function, we examined the phosphorylation status of AMPK Thr-172 and the phosphorylation status of ACC at Ser-79 after treatment with WJPs for 24 h (Fig. 5A, B). The amount of p-AMPK significantly decreased in Caco-2 cell after stimulation with TNF-α. WJPs (100 μg/mL) significantly reversed the decrease. To further determine whether the facilitating effect of WJPs on barrier function involves the AMPK pathway, we compared the effect of WJPs on TER in Caco-2 cell monolayers in the presence or absence of compound C, a specific

inhibitor of AMPK (Fig. 5C). As expected, incubation with WJPs at 100 μg/mL for 24 h led to significant increases in TER in the Caco-2 cell monolayers. However, the effect was abolished by the presence of 10 mmol/L of compound C (Sigma).

3.5 WJPs reduced colonic mucosal permeability in TNBS-induced experimental colitis

As shown in Figure 6, there was a significant increase in permeability to Evan's blue in rats from the model group compared with the normal control group ($p < 0.05$). In contrast, treatment with WJPs resulted in a significant reduction of permeability in rats with TNBS-induced colitis.

3.6 WJPs protected the integrity of the epithelial barrier in TNBS-induced colitis

One major component of the intestinal barrier is the formation of tight junctions (TJ) between epithelial cells. We determined whether the expressions of 4 major tight junction proteins (occludin, claudin-1, claudin-4, and ZO-1) in TNBS-induced colitis were regulated by WJPs using western blots. As shown in Fig. 7 and Fig. 8, occludin, claudin-1, claudin-4 and ZO-1 expression was notably down-regulated in the TNBS-treated rats, as compared to the controls. WJPs (80 mg/kg) administration led to a dramatic up-regulation of the expression of these tight junction proteins.

3.7 WJPs effects on AMPK activity in TNBS-induced colitis

A considerable body of evidence indicates that the activities of activation of the AMP-activated protein kinase (AMPK) regulate the integrity of TJ [15]. To determine whether AMPK is involved in the pathway through which WJPs regulates the function of tight junctions, we examined the phosphorylation status of AMPK Thr-172 in TNBS-induced colitis. As shown in Fig.9B, the phosphorylation of AMPK Thr-172 was notably down-regulated in the TNBS-treated rats, as compared to the controls while the amount of p-AMPK increased after the treatment with WJPs. An antibody against p-ACC at Ser-79 was utilized to determine the ACC phosphorylation at the same time. The levels of ACC phosphorylation were also increased after WJPs treatment, which was in good agreement with the corresponding changes in the levels of AMPK phosphorylation, reflecting an increase in AMPK activity.

4. Discussion

In our present study, the encouraging findings have indicated for the first time that: 1) WJPs, wild jujube (*Ziziphusjujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou*) sarcocarp polysaccharides, protect against experimental colitis by enhanced intestinal barrier function; 2) the effect of WJPs on the intestinal barrier is mediated by the regulation of the assembly of tight junctions involving the activation of AMPK.

Intestinal barrier function is critical for the pathogenesis of gastrointestinal disorders to maintaining mucosal homeostasis[33]. Although the etiology and pathogenesis of IBD are incompletely elucidated, the main feature of the IBD is the overproduction of pro-inflammatory cytokines, as well as the epithelial barrier dysfunction[37]. A growing body of evidence indicates that pro-inflammatory cytokines play a key role in the induction of barrier defects during IBD [38, 39]. Increased paracellular permeability in turn enhances antigenic exposure to underlying immune cells, further compromising barrier function [40]. Several mechanisms can be responsible for this pro-inflammatory cytokines-induced disruption, including a down-regulation of the expression of TJ proteins. Here, for the first time, we show the protective effects of WJPs against experimental colitis by enhanced intestinal barrier function.

TNBS-induced colitis is widely used as a disease model for the evaluation of the effects of various drugs because of its similarity to Crohn's disease. In this study, we demonstrated a protection against colitis induced by TNBS in rats with WJPs treatment, exhibiting improved DAI scores, colon length, wet weight, histological damage and production of pro-inflammatory cytokines. To further explore mechanisms responsible for the observed beneficial effects of WJPs on TNBS-induced colitis, we analyzed the effect of WJPs on the anti-inflammatory. IBD is associated with excessive generation of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) which amplifies the inflammatory cascade by triggering the generation of other proinflammatory cytokines and enhancing the recruitment of macrophages and neutrophils [41]. However, there is no effect of WJPs on the production of pro-inflammatory cytokines (TNF-α) in the macrophage. It has

reported that pro-inflammatory cytokines may be a pathophysiologically relevant regulator of intestinal epithelial tight junction permeability [7, 8]. In addition, the recovery in the epithelial barrier function may result indirectly in a decrease in cytokine production[42]. The decrease of pro-inflammatory cytokines and the leukocyte invasion in TNBS-induced colitis with WJPs treatment may relate to epithelial barrier function, but not anti-inflammatory.

Thus, we analyzed gut epithelial barrier function, a primary predisposing factor for the incidence of IBD [5]. The Caco-2 cell monolayer was used as an *in vitro* model of the intestinal barrier [43]. TNF, a critical mediator of Crohn's disease, has been recognized as a regulator of tight junction permeability [34, 35]. By using this model, the research shows that the ability of WJPs to alleviate the increased paracellular permeability exposed to TNF-α as measured by increases in TER and decreases in FD-4 permeability. The intestinal barrier is organized by the interaction of different barrier components including intercellular TJ proteins[44]. The assembly of the TJ proteins is very important for the formation and maintenance of the barrier function[45].TJ proteins is dynamically regulated and regulate the passage of ions and small molecules through the paracellular pathway and restrict the diffusion of membrane proteins between the apical and basolateral compartments [15]. Proteins constituting the TJ complex include the transmembrane proteins occludin, claudin family members and junctional adhesion molecule (JAM), and linker proteins such as zonula occludens 1 (ZO-1) that affiliate with the underlying actin cytoskeleton [40, 46]. The states of occludin, claudins and ZO-1 are vital for maintaining the TJ structure and function [35, 47]. We speculated that the effect of WJPs on the epithelial barrier may be mediated by regulating the expression of TJ proteins. In the present study, the expressions of 4 major TJ proteins (occludin, claudin-1, claudin-4, and ZO-1) in TNBS-induced colitis were regulated by WJPs. Thus, the effect of WJPs on the epithelial barrier could be mediated by regulating the expressions of tight junction proteins (TJ) between epithelial cells.

A considerable body of evidence indicates that the activities of activation of the AMP-activated protein kinase (AMPK) regulate the integrity of TJ. The tight junction

assembly is impaired when AMPK activity is downregulated [15, 21]. AMPK plays an important role in maintaining cellular energy balance [48]. The activity of AMPK will phosphorylate downstream regulatory proteins and enzymatic effectors to up-regulate ATP-producing catabolic pathways and down-regulate ATP-consuming processes [49, 50]. AMPK activity has a specific role in TJ-related epithelial morphogenesis which is related to cingulin– microtubules association [51]. Moreover, AMPK activation protects TJ proteins via the suppression of ROS in lipopolysaccharide-induced the barrier dysfunction [52]. Polysaccharides from Radix hedysari can activate AMPK pathway in non-alcoholic fatty liver disease[53]. However, there is no report about the AMPK pathway activation by ploysaccharides in intestinal epithelial cells. In our study, the increases of p-AMPK and p-ACC in Caco-2 cells and in TNBS-induced colitis by WJPs imply that WJPs can activate AMPK in intestinal epithelial cells either directly or indirectly. Furthermore, compound C, a cell-permeable competitive specific AMPK inhibitor, inhibits the WJPs-induced activation of AMPK. The effect of WJPs on the increases in TER and decreased in FD-4 permeability in Caco-2 cell after stimulation with TNF-α is abolished by compound C. These data further indicate that WJPs enhances intestinal barrier function by facilitating tight junction assembly and the regulatory effect of WJPs on the assembly of the tight junction proteins depends on the activation of AMPK.

In summary, the aim of the present study was to assess the therapeutic potential of polysaccharides from wild jujube in 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colitis, which resembles Crohn's disease in humans. The results of this study demonstrate that the polysaccharides from wild jujube regulation of barrier function in the Caco-2 cell monolayer model and in 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colitis. Additionally, the effect of polysaccharides from wild jujube on the regulation of intestinal barrier function is related to regulation of the assembly of tight junctions involving the activation of the AMPK.

Acknowledgement

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Figure legends.

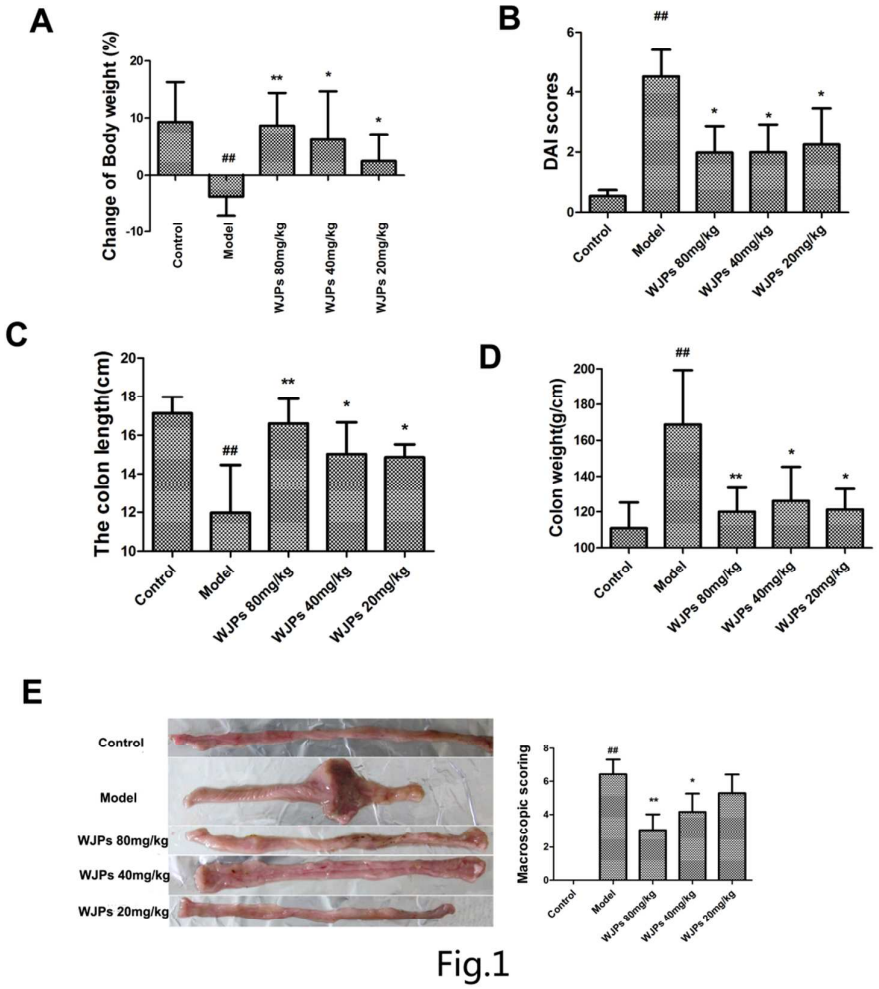

Fig.1

Fig. 1. Protective effects of WJPs against TNBS-induced colitis in rats. Measurements were performed 14 days post TNBS instillation and WJPs was administered for 10 days starting 3 days after colitis induction. (A) Change of Body weight (%) (B) Disease activity index. (C) Colon length. (D) Colon weight (Colon weight/length, g/cm). (E) Macroscopic appearance (left panel) and macroscopic scores of each group was evaluated (right panel). All data were expressed as mean ± SD (n= 8). ##P<0.05 versus control, *P < 0.05 versus vehicle, **P < 0.01 versus vehicle.

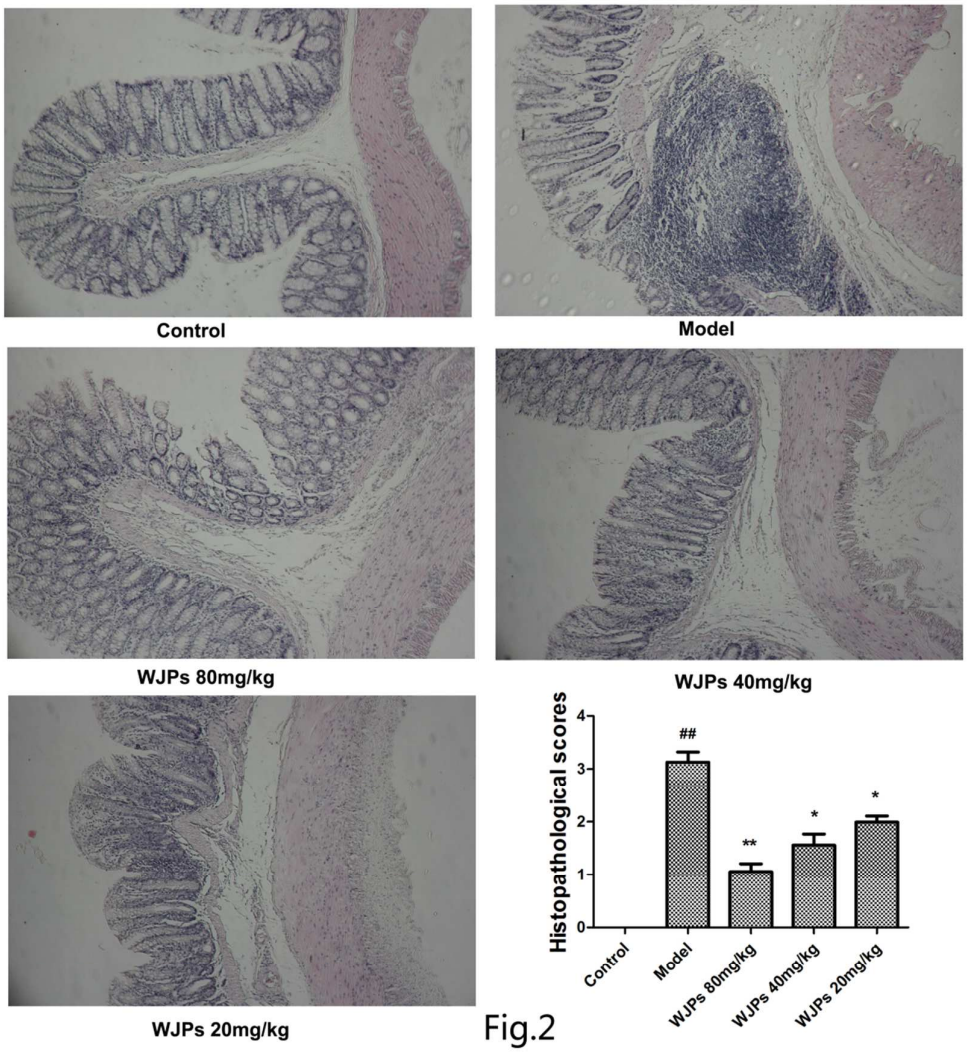

Fig. 2. Representative photomicrographs of HE-stained paraffin-embedded sections of colon tissue obtained from each group (×100). Measurements were performed 14 days post TNBS instillation and WJPs was administered for 10 days starting 3 days after colitis induction. Histological appearance of tissue in each group was scored. All data were expressed as mean ± SD (n= 4). ##P<0.05 versus control, *P < 0.05 versus vehicle, **P < 0.01 versus vehicle.

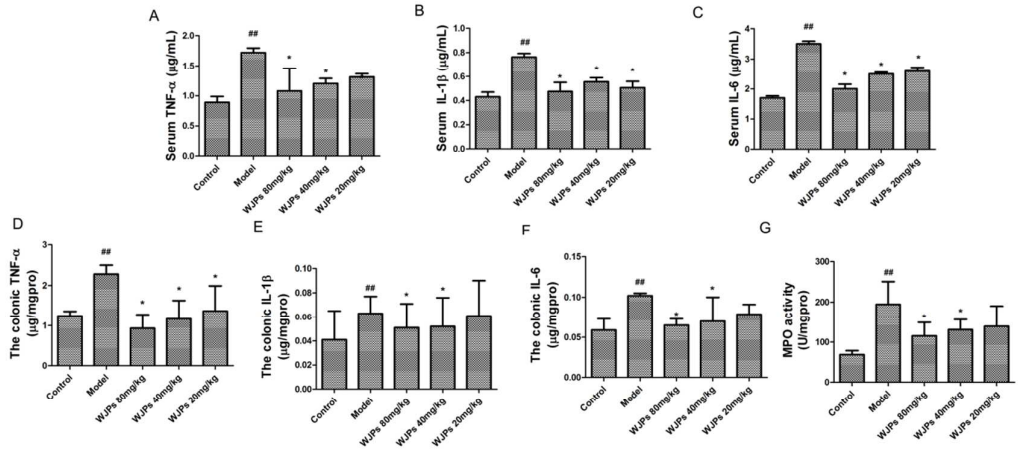

Fig.3

Fig. 3. WJPs modulate inflammatory cytokines and leukocyte invasion in serum or colon of rats with TNBS-induced colitis. Measurements were performed 14 days post TNBS instillation and WJPs was administered for 10 days starting 3 days after colitis induction. Levels of tumor necrosis factor-α; TNF-α (A), interleukin-1β; IL-1β (B) and interleukin-6; IL-6 (C) in serum were determined by ELISA. Levels of TNF-α (D), IL-1β (E), IL-6 (F) and myeloperoxidase (G) in colon were determined. All data were expressed as mean ± SD (n= 8). ##P<0.01 versus control, *P < 0.05 versus vehicle.

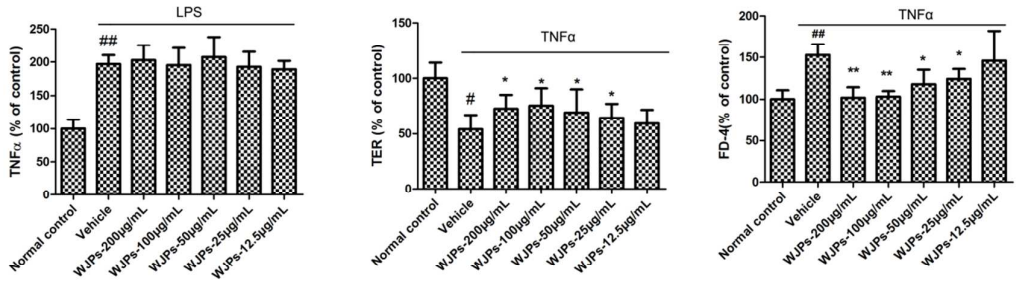

Fig.4

Fig. 4. Effects of WJPs on the TNF-α release from RAW264.7 cells stimulation with LPS (A) and the effects of WJPs in Caco-2 cells stimulation with TNF-α (B, C). LPS (100 ng/mL)-stimulated RAW264.7 were incubated with or without WJPs (12.5, 25, 50, 100 or 200 μg/mL) for 48 h. TNF-α (20ng/mL)-stimulated Caco-2 cell monolayers were incubated with or without WJPs (12.5, 25, 50, 100 or 200 μg/mL) for 48 h. Paracellular flux (B) was determined using Inulin-FITC probes in Caco-2 cell monolayers treated similarly for 48 h. All data were expressed as mean ± SD (n= 3). #P<0.05 versus control, ##P<0.01 versus control, *P < 0.05 versus vehicle.

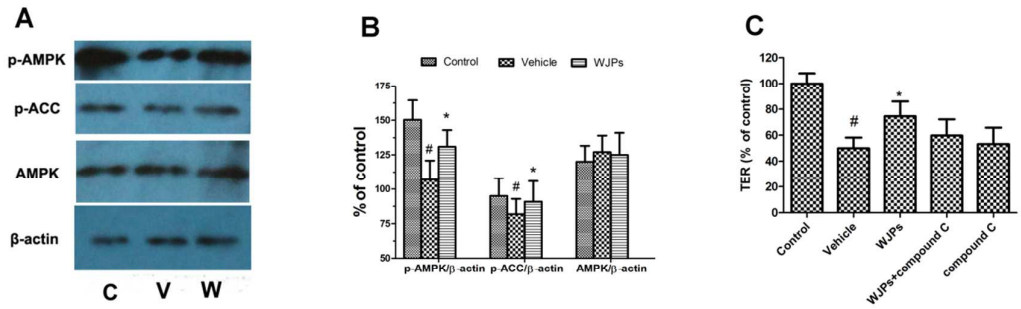

Fig.5

Fig. 5. Effects of WJPs on AMPK pathway in Caco-2 cells stimulation with TNF-α. TNF-α (20ng/mL)-stimulated Caco-2 cell monolayers were incubated with or without WJPs (100 μ g/ mL) for 48 h. The western blots analysis for p-AMPK, total AMPK and p-ACC in Caco-2 cells stimulation with TNF-α (A, B). TER (C) was measured at 48 h after the administration of WJPs in the presence or absence of compound C, a specific inhibitor of AMPK. All data were expressed as mean ± SD (n= 3). C, control; V, vehicle; W, 100 μ g/ mL; #P<0.05 versus control, ##P<0.01 versus control, *P < 0.05 versus vehicle.

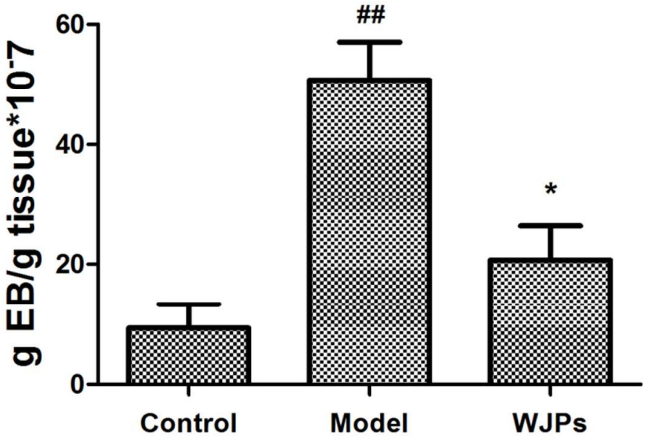

Fig.6

Fig. 6. Effects of WJPs on colonic mucosal permeability. The significant increase in permeability to Evan's blue in rats with TNBS-induced colitis was decreased significantly following treatment with WJPs. All data were expressed as mean ± SD (n= 6). #P<0.05 versus control, ##P<0.01 versus control, *P < 0.05 versus vehicle.

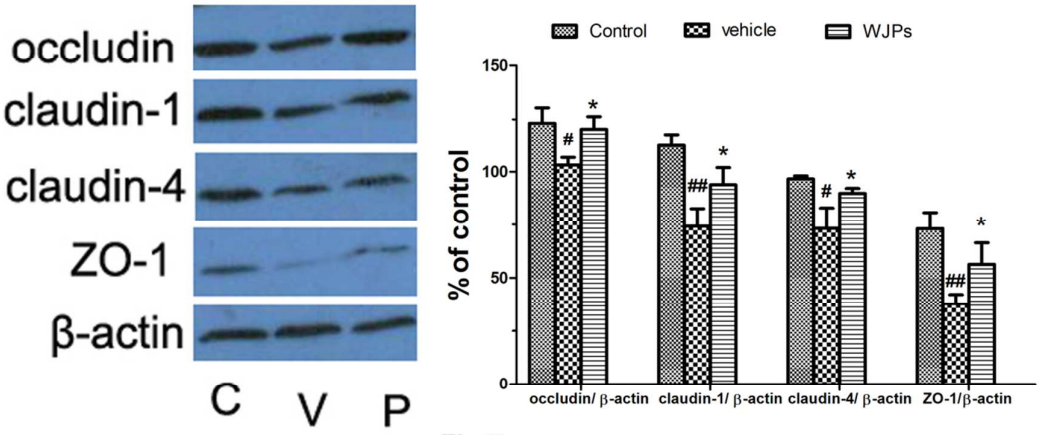

Fig.7

Fig. 7. Effects of WJPs on the expressions of tight junction proteins in rats with TNBS-induced colitis. Measurements were performed 14 days post TNBS instillation and WJPs was administered for 10 days starting 3 days after colitis induction. Analysis of TJ proteins using western blots. All data were expressed as mean ± SD (n= 4). C, control; V, vehicle; W, WJPs 80mg/kg; #P<0.05 versus control, ##P<0.01 versus control, *P < 0.05 versus vehicle.

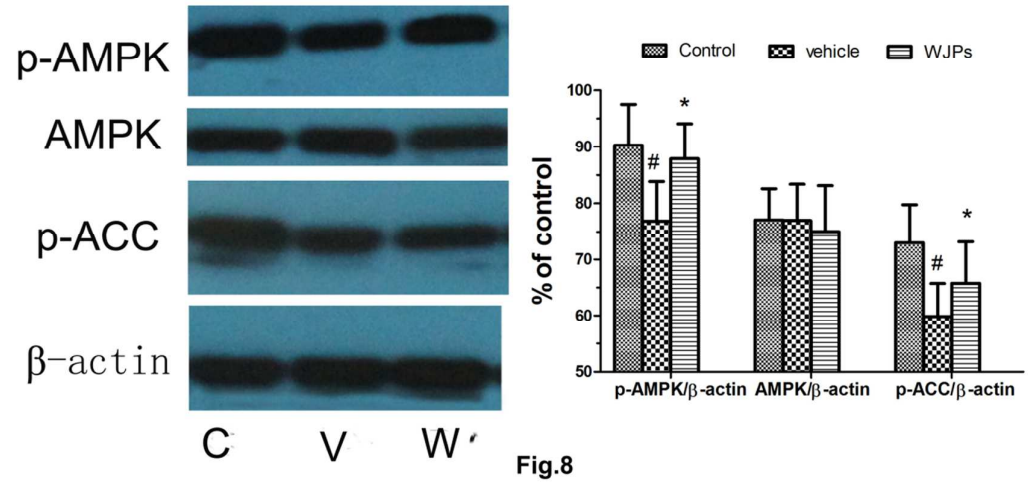

Fig.8

Fig. 8. Effects of WJPs on AMPK pathway in colon of rats with TNBS-induced colitis. Measurements were performed 14 days post TNBS instillation and WJPs was administered for 10 days starting 3 days after colitis induction. The western blots analysis for p-AMPK, total AMPK and p-ACC in colon of rats with TNBS-induced colitis. All data were expressed as mean ± SD (n= 4). C, control; V, vehicle; W, WJPs 80mg/kg; #P<0.05 versus control, ##P<0.01 versus control, *P < 0.05 versus vehicle.

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