The Ameliorating Activity of *Portulaca oleracea* L. Polysaccharide on the UC Mouse Model Induced by DSS

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**Abstract**

Ulcerative colitis (UC) is the chronic non-specific intestinal inflammatory disease. The purpose of this study is to establish a UC mouse model and explore the improvement effect of *Portulaca oleracea* L. polysaccharide (POL-P) on the UC experimental model. Dextran Sulphate Sodium Salt (DSS) concentration screening for establishing UC mouse model was used 2% DSS, 3% DSS and 4% DSS, respectively. Disease activity index (DAI) was recorded daily. Mice were sacrificed at the end of the experiment. The length of the colon and morphological structure of colonic tissue were analyzed. To further evaluate the safety of POL-P, the morphology analysis of liver and kidney tissue sections was performed. The results showed that 4% DSS was the optimal modeling concentration. POL-P had obvious prevention and treatment effects on UC mice. And POL-P has no toxic side effects on the liver and kidney tissues of mouse. The experimental results provide a theoretical basis for developing POL-P into a new drug with high efficiency and low toxicity for the prevention and treatment of UC.

**Keywords:** Ulcerative colitis; *Portulaca oleracea* L. polysaccharide; Dextran Sulphate Sodium Salt; Disease activity index

**INTRODUCTION**

Ulcerative colitis (UC) is the chronic non-specific intestinal inflammatory disease [1]. The incidence has increased dramatically with the acceleration of life rhythm, the change of diet structure and the improvement of detection level. Moreover, it is not easy to heal, leading to a high incidence of colon cancer [2, 3]. The treatment of UC with western medicine mainly focuses on immunosuppression and anti-inflammation. However, it will cause more adverse reactions because western medicine is used long-term repeated [4]. *Portulaca oleracea* L. is a kind of food and medicine homologous plant with the functions of clearing away heat and detoxification [5]. Clinical studies have found that *Portulaca oleracea* L. has significant efficacy in the treatment of UC, however, its reasonable and safe use is hindered by its unclear scientific connotation [6]. The purpose of this study is to establish a UC mouse model and explore the improvement effect of *Portulaca oleracea* L. polysaccharide (POL-P) on the UC mouse model.

**MATERIALS AND METHODS**

**MATERIALS**

POL-P was obtained from *Portulaca oleracea* L. in our laboratory. The more details for preparation of water-soluble polysaccharide including separation, purification and identification refered to our previous work. Dextran Sulphate Sodium Salt (DSS) was obtained from MP Biomedical (Solon, OH, United States). All other reagents used were of analytical grade.

BALB/c female mice (6-8 weeks) were provided by the Experimental Animal Center of Changchun. The animals were treated according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the Institutional Animal Care and Use Committee of Heilongjiang August First Land Reclamation University.

**DSS concentration screening for establishing UC mouse model**

Forty mice were divided randomly into 4 groups, including control group, 2% DSS group, 3%
DSS group, 4% DSS group, ten mice in each group. Freely drink purified water for three days. On the 4th day, the mice in the control group drink purified water freely, and the mice in the model group drink freely drinking water containing 2%, 3%, and 4% DSS. The mental signs and activity status in each group of mice were recorded every day and scored according to the table below. The final DAI score = (weight loss score + stool trait score + stool blood condition score) / 3.

<table>
<thead>
<tr>
<th>Scoring</th>
<th>Falling in body mass %</th>
<th>Stool status</th>
<th>Blood in the stool</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>normal</td>
<td>negative (-)</td>
</tr>
<tr>
<td>1</td>
<td>0~5</td>
<td>fall in between</td>
<td>Fall in between</td>
</tr>
<tr>
<td>2</td>
<td>5~10</td>
<td>semi-sparse</td>
<td>occult blood (+)</td>
</tr>
<tr>
<td>3</td>
<td>10~15</td>
<td>fall in between</td>
<td>fall in between</td>
</tr>
<tr>
<td>4</td>
<td>≥15</td>
<td>loose stool</td>
<td>gross bloody stool</td>
</tr>
</tbody>
</table>

**HE staining**
Mice were sacrificed and the liver, kidney, colon tissues were collected. The intestinal contents were rinsed with PBS solution. After rinsing, both ends of the colon tissue were repaired with a blade to remove the rolled and damaged parts during rinsing, and fixed with neutral formalin solution for 24 h. The paraffin sections were prepared by the following procedures, such as fixation, trimming, dehydration, transparency, paraffin impregnation, embedding and sectioning, and then HE staining was performed. The tissue changes of liver, kidney and colon were observed under a microscope.

**Preventive and therapeutic effects of POL-P on UC mice**
Sixty mice were fed normally for one week and randomly divided into 6 groups with 10 mice in each group. The positive antimicrobial peptide group was given with 0.2 g/mL, 0.2 mL/10 g/time, once a day by intragastric administration. POL-P low-dose group (0.2 g/mL), POL-P medium-dose group (0.4 g/mL) and POL-P high-dose group (0.8 g/mL) were given by intragastric administration at 0.2 mL/10 g/time, once a day, respectively. The experiment lasted for 12 days. On the first 5 days of the experiment, mice in the control group and model group were given normal saline by intragastric administration while drinking normal water, and the positive group and POL-P low-dose, medium-dose and high-dose groups were given intragastric administration at the above dose. On the 6th day of the experiment, except the control group, the UC mice model was established with 4% DSS water solution instead of drinking water in other groups, and continued to be gavage until the last day of the experiment according to the method of the previous 5 days. Mental signs and activity status of mice in each group were recorded every day and scored according to the following table, and the final DAI score = (body weight loss score + fecal traits score + stool blood condition score) / 3.

**RESULT**

**DAI score results**
The results of DAI score were showed in Figure-1. The fecal particles were clear and the degree of soft and hard was suitable in the control group (Figure 1A), while the feces was soft and severe hematochezia and even serious anal bleeding were observed in the 4% DSS group (Figure 1B). DAI score results showed that the control group had a lower score, while 4% DSS group had a higher score. DAI score results showed that it was a higher score in 4% DSS group, compared with control group (Figure 1C, P<0.0001).
Colon changes in mice

After the animals were sacrificed, the abdominal cavity was opened by cross method, and the whole intestine was carefully dissected for length measurement. The results showed in Figure-2, as a result of intestinal mucosal injury resulting in mucosal congestion and edema, the intestinal wall was thickened and the length of the intestine was shortened in the 4% DSS group. It is significantly different from the control group.

![Colon length comparison between control group and 4% DSS group](image)

Note: Compared with Control, ★★ P<0.01

Colonic histopathological observation

The results of colonic histopathology were showed in Figure 3. There was no obvious damage of colon tissue in the control group. In the 4% DSS treatment group, the number of goblet cells was decreased, and neutrophils had infiltrated the intestinal lamina propria. The presence of transmural inflammation and ulceration in the intestine indicated that the integrity of the intestinal epithelium had been severely compromised. Further, the colonic lesion has reached the submucosa with crypt glandolysis. Histopathological scores showed that there was a significant difference between the 4% DSS group and the control group.

![HE staining results and histopathological score](image)

Note: Compared with Control, ★★★★ P<0.0001

The effect of POL-P on DAI score of UC mice

The three basic indexes of weight loss, fecal viscosity and fecal occult blood were combined to evaluate the DAI score of mice. As showed in Figure-4, after DSS intervention, DAI index of model group, positive group and low, medium and high dose groups were significantly increased. The DAI score in the model group was the highest compared with other group mice. And the DAI index showed a downward trend with the increase of POL-P concentration. It suggested that POL-P had a certain therapeutic effect on UC mice, and the high-dose performed the best effect.

![The effect of POL-P on the DAI score of the UC mouse model](image)

Note: compared with Control, ★★★★ P<0.0001; compared with DSS, ★★★ P<0.05, ★★★★ P<0.01; compared with POL-P 800 mg and 400 mg, ★★★★ P<0.01.
Effect of POL-P on colon length in UC mice
The effect of POL-P on colon length in UC mice was showed in figure 5. The average length of the colon in mouse of the model group was the shortest, and the length was elevated with the increase of POL-P concentration.

Fig-5: Effect of POL-P on colon length in UC mice
Note: Compare with Control, *P<0.05; compare with DSS, **P<0.01, ***P<0.001

Effect of POL-P on colonic tissue of UC mice
The effects of POL-P on the morphological structure of colonic tissue were showed in Figure-6. The colonic mucosal epithelium was intact, and the glands in the lamina propria were normal in control group. In addition, there were many goblet cells in the mucosal epithelium, and no degenerative necrosis, no edema in the submucosa, and no infiltration of inflammatory cells were observed in control group. The obvious symptoms of ulcerative colitis in intestinal tract were appeared, such as thicken intestinal wall, denatured and necrotic epithelial cells, disappeared crypt, missing goblet cells, and numerous inflammatory cell infiltration in 4% DSS treatment group. The above pathological features were improved by treatment with POL-P. The pathological score in the model group was significantly higher than that of the control group. Compared with model group, pathological scores in POL-P groups were significantly decreased. These results suggested that POL-P could improve the intestinal mucosal injury induced by DSS.

Fig-6: Effects of POL-P on colonic structure of UC mice
Note: Compare with Control, ####P<0.0001; compare with DSS, ***P<0.001, ****P<0.0001

Effect of POL-P on liver and kidney tissue of mice
To further evaluate the safety of POL-P, the morphology analysis of liver and kidney tissue sections was performed. As showed in Figure-7, compared with the control group, the hepatic structure was complete without damage and central vein was in the center. The hepatic cords were arranged radially, and the liver cells were arranged neatly with normal volume, without inflammatory cell infiltration and necrotic cell generation. In addition, the renal tissue structure was normal, and the glomerular and tubular structures were clear and intact (Figure-8). The observation results suggested that POL-P had no obvious toxic effect on liver and kidney of mice.

Fig-7: Effect of POL-P on liver tissue of mice (20×10)
DISCUSSION
Ulcerative colitis is a chronic inflammatory bowel disease caused by multiple factors. It is characterized by high incidence, long course and repeated attacks. Its clinical manifestations are mainly abdominal pain, diarrhea and mucinous pus and bleeding stools [7]. UC tends to occur in the sigmoid colon and rectum, but serious cases can also involve the whole intestine, which seriously affects People's Daily life[9]. Studies have found that patients with inflammatory bowel disease (IBD), which has a disease duration of more than 30 months, has a cancer rate of 45% [8]. The epidemiological data at domestic and overseas show that the prevalence and incidence of UC is an obvious upward trend, and it has been listed as one of the modern refractory diseases by the World Health Organization [9]. In recent years, the incidence of UC in China has been rising and gradually getting younger. At present, the clinical treatment of UC in western medicine is mainly to control acute attacks, relieve colonic inflammation, and thus relieve symptoms of diarrhea, rectal bleeding and abdominal pain. However, these drugs are expensive and have serious side effects. It can not meet the needs of clinical application because it is prone to relapse after drug withdrawal and the treatment effect is not ideal for some refractory cases [10]. Therefore, it is urgent to find a kind of therapeutic drugs with definite curative effect, small toxic and side effects and long-term use.

In recent years, traditional Chinese medicine has a remarkable effect on the treatment of this disease. At the same time, it have many advantages such as low relapse rate, little adverse reaction, reduction of hormone dependence, and short course of treatment which has become a research hotspot. *Portulaca oleracea* is the whole grass of *Portulaca oleracea* L., which has the effects of heat-clearing and detoxifying [11]. *Portulaca oleracea* is rich in nutrition and is one of the wild medicinal materials with the same origin of medicine and food as recognized by the Ministry of Health of China [12, 13]. As a traditional gastrointestinal drug, it has been proved to be effective in the treatment of UC by years of clinical application [14, 15]. However, studies on *Portulaca oleracea* in the treatment of UC mostly focus on extractum, water decoction and crude extract, and the effect of active ingredient on UC and its mechanism of action have not been reported yet. In this study, sodium dextran sulfate was used to establish a UC mice model. Polysaccharide, the active ingredient of *Portulaca oleracea* was prepared in our laboratory and was used to treat UC model mice with different doses. The improvement effect of POL-P on UC mice was determined by analyzing DAI, pathological damage of colon, inflammatory cell infiltration degree, villus length and the depth of crypt in each group. The results of this study provide a theoretical basis for revealing the clinical application of POL-P in the treatment of UC.

CONCLUSION
POL-P, the active ingredient of *Portulaca oleracea* L. had obvious prevention and treatment effects on UC mice induced by DSS. And POL-P has no toxic side effects on the liver and kidney tissues of mouse. The experimental results provide a theoretical basis for developing POL-P into a new drug with high efficiency and low toxicity for the prevention and treatment of UC.

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