SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Life Science and Technology

Ultrasonic and Microwave Co-Extraction Optimization of Polysaccharide of *Portulaca oleracea* L. Technology and its Effect on Ulcerative Colitis

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DOI: 10.36347/sasjm.2021.v07i12.006 | **Received:** 03.11.2021 | **Accepted:** 07.12.2021 | **Published:** 12.12.2021

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Abstract **Original Research Article**

The extraction conditions of polysaccharides from *Portulaca oleracea* L.（POP） were optimized and its effects on the colonic mucosa of mice with ulcerative colitis were studied. In this study, POP were extracted by ultrasonic and microwave synergism. The extraction process of POP was optimized by a single factor experiment and orthogonal design. HE staining was used to evaluate the pathological changes of colon tissues in mice. The results showed that the optimal extraction conditions were as follows: extraction time was 550 s, microwave power was 40 W, solid-liquid ratio (W/V) was 1:35, and extraction times were 3. Under these conditions, the extraction rate of POP was 3.80%. POP could improve the disease state of UC mice, and the high dose group could significantly reduce the DAI index. The results of the HE experiment showed that POP could reduce inflammatory infiltration and tissue damage of the colon in UC mice. This study provided a theoretical basis for the preparation of POP and its clinical application in the treatment of ulcerative colitis.

Keywords: *Portulaca oleracea* L. polysaccharide (POP); Microwave ultrasonic co-extraction; Ulcerative colitis; HE dyed.

INTRODUCTION

Ulcerative colitis (UC) is a common and difficult disease of the digestive system [1]. In recent years, with the deterioration of the social environment, the acceleration of the pace of life, the change of diet structure, and the continuous improvement of detection level and other factors, the incidence and prevalence of colon cancer have increased by more than 10 times in the past five years, and the risk of inducing colon cancer has been increasing along with the delay, seriously endangering health [2-4]. Modern medicine in the treatment of UC with corticosteroids and nonsteroidal anti-inflammatory drugs and immunosuppressants, inhibit the excessive release of inflammatory cytokines, reduce the reaction and mucosa damage to achieve therapeutic purposes [5-8], but these drugs are expensive, and stop drug-resistant, easy to break out repeatedly, easy to cause viral infections such as shortcomings and the insufficiency [9-11], the treatment effect is not satisfactory, unable to meet the needs of clinical application. The heat-clearing and detoxification method is one of the methods widely used in the treatment of UC in contemporary Chinese medicine and has achieved good efficacy [12-14]. *Portulaca oleracea* L. was first recorded in the "Shennong Herbal Classic", which functions in clearing heat and detoxification, cooling blood, and stopping bleeding, and is used for hot poison dysentery. As a long-honored medicinal and food plant, modern literature reports that it has pharmacological effects such as anti-inflammatory, antibacterial, pain relief, and promoting healing[15-16]. In long-term clinical studies, *Portulaca oleracea* L. treated UC significantly [17-19], but its active component and mechanism of action need to be further explored.

Portulaca oleracea polysaccharide (POP) is one of the main active components of Chinese medicine purslane, as well as other plant polysaccharides, can increase the body's immune function, anti-tumor, anti-oxidation, anti-aging, scavenging free radicals, and other functions[20, 21], its extraction method are mainly water decoction, enzyme formulation, ultrasonic extraction, microwave extraction, etc., in which ultrasonic microwave synergistic extraction time, high efficiency, convenient operation [22-24]. Membrane dialysis technology can selectively pass through the raw

material components with a certain potential differences, pressure or concentration difference, so as to achieve the separation of large and small molecules, and the operation is simple and easy to automate.

In this experiment, the orthogonal design was adopted to optimize the ultrasonic and microwave co-extraction technology of POP, the protein was deproteinized by trichloroacetic acid-n-butanol method, the small molecular impurities in crude polysaccharides were removed by dialysis by membrane separation technology, HE staining was used to evaluate the effect of POP on the pathological morphology of colon mucosa of UC mice, so as to provide a preliminary theoretical basis for the development and utilization of UC by *Portulaca oleracea* L.

1. Instruments and materials

1.1 Instruments

Ultrasound-microwave co-extractor (CW-2000): Xintuo microwave sample test technology co. LTD; Analytical balance (FA2014B) : Shanghai yueping scientific instrument co., LTD; Ultrasonic cleaner (KQ-600e): Kunshan ultrasonic instrument co., LTD; Ultraviolet spectrophotometer (6715): JENWAY; High speed multi-function grinder (RH-600A): Zhejiang ronghao industry and trade co., LTD; Digital display constant temperature water bath boiler (HH-6): jintan chengxi zhengrong experimental instrument factory; Centrifuge (80-2) : jintan chengxi zhengrong experimental instrument factory.

1.2 Reagent

Phenol (Shenyang huadong reagent factory); Petroleum ether, anhydrous ethanol, acetone (Liaoning quanrui reagent co., LTD.); Concentrated sulfuric acid, n-butanol, trichloroacetic acid, xylene, ammonia, hydrochloric acid (Tianjin kemi ou chemical reagent co., LTD.), sodium chloride, potassium chloride, disodium phosphate dodecahydrate, potassium dihydrogen phosphate (yatai chemical co., LTD.), were all analysis-pure.Sephadex g-150 (Sigma, USA); Dialysis bag (MW5000, Bromma);DSS (molecular weight 50 000, kameisu biotechnology co., LTD.). Bouin's solution (saturated picric acid, formalin), phosphate buffer (PBS), double steamed water, 0.75% normal saline, sterilized cotton ball laboratory homemade.

1.3 MATERIALS

Portulaca oleracea L. was purchased from Anhui daoyuantang traditional Chinese medicine decoction pieces co., LTD. Anhydrous glucose standard was purchased from Hefei bomei biotechnology co., LTD. Sulfasalazine enteric-soluble capsule (SASP) was purchased from Guangdong Qiangji pharmaceutical co. LTD.

2. METHOD

2.1 Preparation of standard curve

Weigh 250 mg of glucose dried at 105 ℃ with constant weight, place it in a 250 mL volumetric bottle, and prepare 1 mg/mL glucose solution. Extract 10 mL from it, place it in a 100ml volumetric bottle, and prepare a 0.1mg /mL glucose solution. Then extract 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL of the solution into a 10 mL volumetric flask, and prepare it into standard solutions of 0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/mL, 0.06 mg/mL, and 0.07 mg/mL.1 mL of the standard solution of each concentration was placed in the test tube, and 1 mL 5% phenol solution and 5 mL concentrated sulfuric acid were added in turn. The solution was shaken and heated in boiling water for 15 min. After the solution was reduced to normal temperature, the absorbance value was measured at 480 nm. With absorbance value as ordinate (y) and standard glucose concentration as abscissa (x), linear regression was performed by the least square method.

2.2 Ultrasonic and microwave co-extraction technology of POP

Dry *Portulaca oleracea* L. was taken, and the dust and impurities on the surface were removed by screening. Add petroleum ether to reflux twice according to 1:10 (g: mL) feed-liquid ratio, dry at low temperature for 2 hours each time, then reflux twice according to the same feed-liquid ratio with 95% ethanol, dry and reserve for 2 hours each time. The degrease *Portulaca oleracea* L. was extracted with distilled water as a solvent, and ultrasonic and microwave-assisted extraction was performed according to certain temperature, solid-liquid ratio, extraction time, etc. After vacuum decompression and concentration of the filtrate, 4 times the volume of anhydrous ethanolization was added, which was placed overnight in a 4 ℃ refrigerator and centrifuged. The precipitation was washed with 95% ethanol, anhydrous ethanol, and acetone for several times, and then freeze-dried to obtain the crude polysaccharides.

2.3 The extraction rate of POP was calculated

Dry *Portulaca oleracea* L. coarse powder was extracted by ultrasonic and microwave co-extraction according to its specific conditions, and the absorption value y was measured by phenol-sulfuric acid method at the wavelength of UV spectrophotometer at 480 nm. The y value was substituted into the standard glucose curve and the content of polysaccharides in the sample was obtained by mathematical calculation.

K（%）=m/M×100%,

Therein: K is the extraction rate of polysaccharide, %; m is the content of polysaccharide in the sample, mg; M is the quality of the drug, mg.

2.4 Single-factor and orthogonal experiment for extraction of POP by ultrasonic microwave

According to the 2.2 technological processes, 2.0 g of crude powder of drug was weighed as the quality of the crude drug used in each experiment, and the effects of extraction time, microwave power, solid-liquid ratio, and extraction times on the extraction rate of polysaccharides were investigated. Based on a single-factor experiment, the extraction rate of polysaccharides was taken as the index to investigate the effects of extraction time (A), microwave power (B), solid-liquid ratio (C), and extraction times (D) on the extraction rate of polysaccharides by an orthogonal experiment of L_9 (4^3).

2.5 Purification technology of POP

The protein in crude polysaccharides was removed by a trichloroacetic acid-n-butanol method. The polysaccharides were dissolved in a proper amount of distilled water, placed in a separating funnel, and an equal volume of trichloroacetic acid-n-butanol (trichloroacetic acid: n-butanol =1:20) was added. The polysaccharides were shaken, stood, and stratified until no milky denatured protein was separated out.

Membrane separation technology was used to remove small molecular impurities in polysaccharides. The polysaccharide solution with protein removed was concentrated to an appropriate concentration and placed in a dialysis bag. The two ends were fixed with dialysis clamps and placed in a large beaker. Distilled water was added to suspend the dialysis bag, and magnetic stirrers were used to stir slowly. Change the water every 12 hours for three times.

2.6 Study on the effect of POP against UC

2.6.1 Grouping, administration method and preparation of UC model

Sixty Kunming mice were randomly divided into 6 groups after 1 W of adaptive feeding: blank control group, model control group, positive control group (Sulfasalazine, SASP, 300 mg·kg^{-1}), and POP group with low, medium and high dose (100, 200 and 400 mg·kg^{-1}), with 10 mice in each group. On the first day, except for the control group, all mice were given drinking water containing 2% DSS. After 6 days of free drinking, normal drinking water was replaced and restored for 14 days. On the 21st day, DSS drinking water was repeated for 6 days and recovery for 4 days. After the third DSS drinking water for 6 days, it recovered for 4 days, and the whole modeling process was 40 days in total. At the same time of model establishment, the POP group was given intragastric administration (0.2 mL/10g) every day. The dose of SASP was converted according to "Experimental Animals and Animal Experiment Technology", and the dose concentration was 15 mg/mL. Model control group and blank control group were given intragastric administration of distilled water in the corresponding volume.

2.6.2 Sample collection and processing

On the 50th day of the experiment, the mice were sacrificed by removing the neck, the abdominal cavity was opened by the cross method, and the colonic mucosa was carefully removed. The damage degree of the colonic mucosa was observed and its length was measured accurately. Intestinal contents were removed with normal saline, 0.5cm was cut from each end of the colon, and Bouin's fluid was fixed and stored for reserve.

2.6.3 Disease Activity Index (DAI) score

From the first day of model establishment, the bodyweight of the mice was measured every day, and the state, hair, feces and diet of the mice were observed and recorded in detail. The score was based on the DAI scoring criteria developed by Murano *et al.* [25]. The specific formula is DAI=(decreased body mass fraction + stool trait fraction + blood fraction)/3.

2.6.4 HE staining of colonic tissue

The colon tissues were fixed and preserved with Bouin's fluid, routine paraffin sections and HE staining was used to observe the morphological changes of the mouse colon under an optical microscope.

3. RESULTS

3.1 Standard curve

The standard curve for the determination of polysaccharide content was drawn (Figure 1), and the regression equation was as follows: y=9.8637x-0.0276, r=0.9994, with good linearity in the range of 0.01~0.07mg/mL.

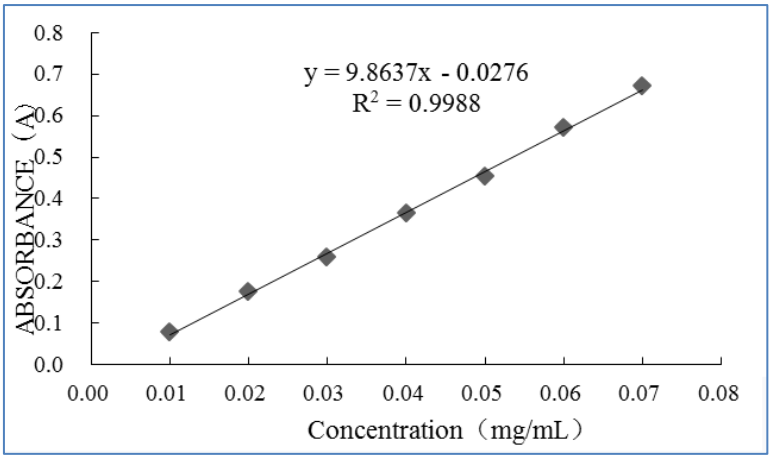

Fig-1: Glucose standard curve

3.2 Single-factor experiment results of ultrasonic microwave synergistic extraction of POP

3.2.1 Effects of ultrasonic on/off on the extraction rate of POP

The initial temperature was set at 45 ℃, the solid-liquid ratio was 1:30 (g/mL), the extraction time was 500 s, the microwave power was 40 W, and the extraction time was 2 times. The effect of ultrasonic on/off on the extraction rate of polysaccharides was investigated. The results showed that the extraction rate of polysaccharides from *Portulaca oleracea* L. was 3.06% when ultrasound was turned on, which was higher than 2.77% when ultrasound was turned off. Therefore, ultrasonic was set to on, and ultrasonic microwave co-extraction was adopted to extract polysaccharides from *Portulaca oleracea* L.

3.2.2 Effect of extraction time on the extraction rate of POP

The initial temperature of 45℃, the solid-liquid ratio of 1:30 (g/mL) and microwave power of 40 W were set to investigate the effects of different extraction times (50 s, 200 s, 350 s, 500 s, 650 s and 800 s) on the extraction rate of POP (Figure 2-a). The extraction rate of POP increased with the extension of extraction time, but when the extraction time exceeded 500s, the extraction rate gradually decreased. Therefore, it was determined that the appropriate extraction time was about 500s.

3.2.3 Effects of microwave power on the extraction rate of POP

The initial temperature was set at 45℃, the solid-liquid ratio was 1:30 (g/mL), and the extraction time was 500 s. The effects of different microwave powers (20 W, 30 W, 40 W, 50 W, and 60 W) on the extraction rate of POP were investigated (Figure 2-b). When the microwave power was less than 40 W, the POP extraction rate increased with the increase of microwave power, and when the microwave power was more than 40 W, the POP extraction rate decreased with the increase of microwave power. Therefore, microwave power of 40 W is suitable.

3.2.4 Effects of solid-liquid ratio on the extraction rate of POP

The initial temperature was set at 45℃, the extraction time was 500 s, and the microwave power was 40 W. The effect of solid-liquid ratio (1:10, 1:20, 1:30, 1:40, and 1:50) on the extraction rate of POP was investigated (Figure 2-c). There was a positive correlation between the extraction rate of POP and the solid-liquid ratio, and when the solid-liquid ratio exceeded 1:40, the increase of the extraction rate tended to be gentle. Therefore, the solid-liquid ratio of 1:40 was more suitable.

3.2.5 Effects of extraction times on the extraction rate of POP

The initial temperature was set at 45 ℃, the extraction time was 500 s, the microwave power was 40 W, and the solid-liquid ratio was 1:40 to investigate the effect of extraction times (1, 2, 3, 4 and 5 times) on the extraction rate of POP (Figure 2-d). There was a positive correlation between the extraction rate of POP and the number of times of extraction. When the extraction was more than 2 times, the increase of the extraction rate tended to be gentle. Therefore, considering the time cost, it was appropriate to determine the extraction number of 2 times.

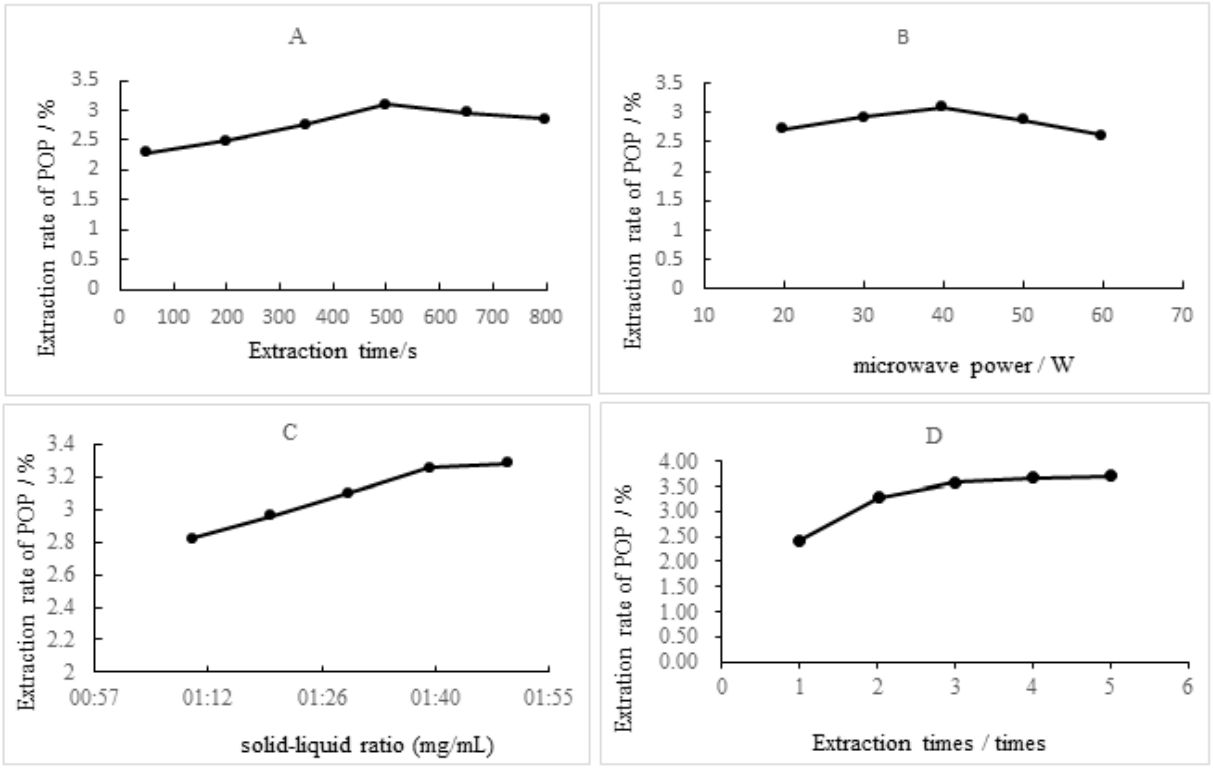

Fig.2 Effect of extraction time, microwave power, solid-liquid ratio, and number of times of extraction on the extraction rate of POP

3.2.6 Optimization of ultrasonic microwave synergistic extraction of POP by orthogonal experiment

On the basis of single-factor experiment, the extraction rate of polysaccharides was taken as an index to investigate the effects of extraction time (A), microwave power (B), solid-liquid ratio (C) and extraction times (D) on the extraction rate of polysaccharides by orthogonal experiment (L_9 (4^3). The experimental design and results were shown in table 1 and 2.

Table-1: Factors and levels of an orthogonal experiment for optimization of POP extraction conditions

Level of factor	Extraction time A/ s	Microwave power B/w	the solid-liquid ratio C/g : mL	Extraction times D/times
1	450	35	1:35	1
2	500	40	1:40	2
3	550	45	1:45	3

Table-2: Results of an orthogonal experiment for optimization of POP extraction conditions

The Test number	A	B	C	D	Extraction rate of POP (%)
1	1	1	1	1	2.50
2	1	2	2	2	3.07
3	1	3	3	3	3.31
4	2	1	2	3	3.11
5	2	2	3	1	2.39
6	2	3	1	2	3.15
7	3	1	3	2	3.53
8	3	2	1	3	3.74
9	3	3	2	1	2.43
K1	8.88	9.14	9.39	7.32	
K2	8.65	9.20	8.61	9.75	
K3	9.70	8.89	9.23	10.16	
R	1.05	0.31	0.78	2.84	

According to the experimental results in table 2, the primary and secondary factors affecting the extraction rate of POP were as follows: extraction times > extraction time > solid-liquid ratio > microwave power. The optimal extraction process of POP was A3B2C1D3, that is, extraction time was 550 s, microwave power was 40 W, liquid-solid ratio (W/V) was 1:35, and extraction times were 3. Three validation tests were carried out according to the optimal combination. The results showed that the average extraction rate of POP was 3.80%, which was close to the results in the orthogonal test table, indicating that the technological conditions were feasible.

3.3 Effect of POP on ulcerative colitis

3.3.1 DAI score of mice

Compared with the model group, the DAI score in all drug treatment groups was lower (p < 0. 05). DAI score of high dose POP group was lower than that of low dose POP group (p < 0. 05). There was no significant difference in DAI score between the high dose POP group and the positive control group (p > 0. 05), as shown in Table 3.

Table-3: DAI scores of each group ($\bar{x}$±s)

group	Number of mice /one	dose/(mg·kg $^{-1}$)	DAI/score
blank control group	10	—	0. 00±0. 00*
model control group (DSS)	8	—	1. 82±0.21
positive control group (SASP)	10	300	0. 89±0. 34*
POP low dose group	9	100	1. 51±0. 27
POP medium dose group	9	200	1. 12±0. 14
POP high dose group	10	400	0. 78±0.13*

Note: Compared with model group, *P＜0. 05。

3.3.2 HE staining of mouse colon tissue

The colonic tissue morphology of mice was observed by HE staining, as shown in figure 3. The damage degree of colonic tissue in each group was in descending order: model DSS group >POP low dose group >POP medium dose group > positive control group >POP high dose group > blank control group. In the blank control group, the colon structure was clear, the mucosal epithelial cells were arranged neatly, and the goblet cells were abundant. The colonic mucosa of mice in the model DSS group was hyperemic and edema, with severe injury, a large number of inflammatory cells infiltrated, the crypt became shallow, and goblet cells decreased or disappeared. Compared with the DSS group, the colon tissue injury in the treatment group was improved in a dose-dependent manner in the low, medium and high dose groups after POP intervention. Among them, the high-dose group had slight mucosal hyperemia and edema and a small amount of inflammatory cell infiltration, and the improvement effect was better than the positive control group.

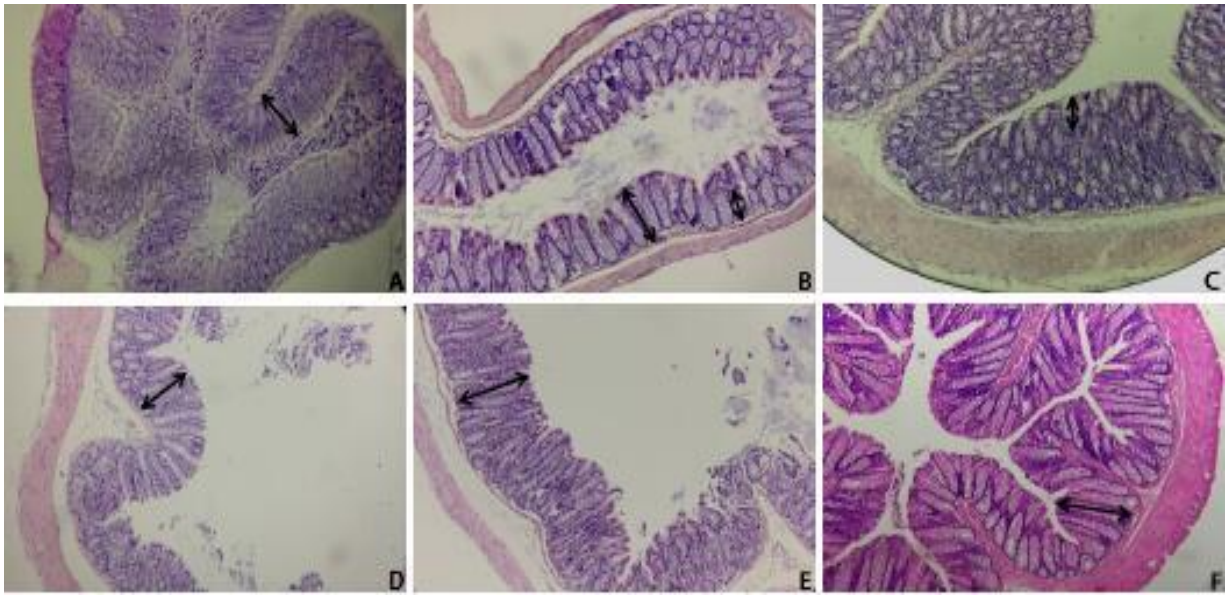

Fig-3: Evaluation on pathological damage of colon tissue in mice by HE staining

Note: A: blank control group, B: model control group; C: positive control group, D: POP low dose group, E: POP medium dose group, F: POP high dose group.

4. DISCUSSION

UC is a complex chronic intestinal disease that is difficult to cure, and the etiology is still not completely clear. The pathological model induced by DSS is the best animal model so far that is consistent with many characteristics of HUMAN UC, such as weight loss, diarrhea, bloody stools, colon edema, colon tissue and pathological changes, etc. A large number of clinical experiments and studies have shown that TCM has unique advantages in the treatment of UC and has achieved great results [26, 27]. UC belongs to the category of "diarrhea", "dysentery" and "bowel fetish" in traditional Chinese medicine, which is mainly caused by disordered diet and exogenous dampness and heat, resulting in steaming of dampness and heat, damage to the spleen and stomach, imbalance of qi and blood, and finally resulting in blood stasis, intestinal collateral damage, meat rot and other symptoms [28]. Therefore, clinical treatment should be in heat detoxification, blood circulation and stasis.

Clinical studies have found that *Portulaca oleracea* decoction or traditional Chinese medicine compound preparation with *Portulaca oleracea* as the primary drug has a significant effect on ulcerative colitis [19]. However, the specific active components of *Portulaca oleracea* are still unclear. Therefore, based on the previous work, this study studied the effective parts of *Portulaca oleracea* in the treatment of UC.

In this study, POP was extracted by ultrasonic and microwave synergic method, deproteinized by trichloroacetic acid-n-butanol method, and small molecular impurities were removed by membrane separation technology. Using POP as the research object, HE staining preliminarily confirmed that after the POP intervention, colonic mucosal tissue damage of UC mice was reduced. These results suggest that POP can relieve the mucosal damage of DSS-induced ulcerative colitis mice and play a role in the treatment of UC, so as to provide a preliminary theoretical basis for the development and utilization of Chinese medicine *Portulaca oleracea* for the prevention and treatment of UC.

ACKNOWLEDGMENTS

This work was supported by Heilongjiang BaYi agricultural university's innovative training project for college students (201810223005).

REFERENCES

1. Bewtra, M., Newcomb, C. W., Wu, Q., Chen, L., Xie, F., Roy, J. A., & Lewis, J. D. (2015). Mortality associated with medical therapy versus elective colectomy in ulcerative colitis: a cohort study. *Annals of internal medicine*, *163*(4), 262-270.
2. Sartor, R. B. (2006). Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nature clinical practice Gastroenterology & hepatology*, *3*(7), 390-407.
3. Jones, S. A., Horiuchi, S., Topley, N., Yamamoto, N., & Fuller, G. M. (2001). The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *The FASEB Journal*, *15*(1), 43-58.
4. Jiang, X. L., & Cui, H. F. (2002). An analysis of 10218 ulcerative colitis cases in China. *World Journal of Gastroenterology*, *8*(1), 158.
5. DONG, Zhi-hua, SUI, Dian-peng, WANG, N.A. (2015). Comparison of the effect of enema of beclomethasone dipropionate and glucocorticoid drugs in treating ulcerative colitis [J]. *Laboratory Medicine and Clinic, 12*(17); 2494-2496.
6. Zhang, Chun-ling, XU Shuo, Wang, Y. (2019). Research Advances on the Treatment of Ulcerative Colitis with Tacrolimus[J]. *Chinese Pharmaceutical Journal*, *7*; 521-525.
7. Colombel, J. F., Sandborn, W. J., Reinisch, W., Mantzaris, G. J., Kornbluth, A., Rachmilewitz, D., ... & Rutgeerts, P. (2010). Infliximab, azathioprine, or combination therapy for Crohn's disease. *New England journal of medicine*, *362*(15), 1383-1395.
8. RONG, Lan. (2016). Application of immunosuppressants in the treatment of inflammatory bowel disease [J]. *Shanghai Medical & Pharmaceutical Journal, 37*(1); 11-15.
9. Tang, S., Yuan, S., Baker, S.S. (2016). Su 1857 Pathological Similarities and Differences Between Crohn's Disease and Ulcerative Colitis Revealed by Systematic Transcriptome Analysis [J]. *Gastroenterology, 150* (4): 571-571.
10. Gao, X. , Zhang F.B. (2012). The potential influence of 5-aminosalicy acid on the induction of myelotoxieity during thiopurine therapy in inflammatory bowel disease patients [J] *Eur J Gastroenterol Hepatol, 24*(8); 958-964.
11. Toruner, M., Loftus Jr, E. V., Harmsen, W. S., Zinsmeister, A. R., Orenstein, R., Sandborn, W. J., ... & Egan, L. J. (2008). Risk factors for opportunistic infections in patients with inflammatory bowel disease. *Gastroenterology*, *134*(4), 929-936.
12. Teschke, R., Wolff, A., Frenzel, C., Eickhoff, A., & Schulze, J. (2015). Herbal traditional Chinese medicine and its evidence base in gastrointestinal disorders. *World Journal of Gastroenterology: WJG*, *21*(15), 4466.
13. Dai, Y. C., Zheng, L., Zhang, Y. L., Chen, X., Chen, D. L., Wang, L. J., & Tang, Z. P. (2017). Jianpi Qingchang decoction regulates intestinal motility of dextran sulfate sodium-induced colitis through reducing autophagy of interstitial cells of Cajal. *World journal of gastroenterology*, *23*(26), 4724.
14. Luo, S. Y., Le, Z., Lv, X. H., & Zhong, Z. G. (2014). Study on effect of total flavonoids of Oldenlendia difflusa on ulcerative colitis and its immunological mechanism. *Zhongguo Zhong yao*

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15. DING, H. W., YAO, J. Q., & SONG, S. J. (2008). Research progress on chemical constituents and pharmacological activities of *Portulaca oleracea* L.[J]. *Journal of Shenyang Pharmaceutical University*, *10*.
16. QIN, X. Z., WANG, D., ZHANG, H. Y., & ZHANG, B. L. (2010). Study on the anti-inflammation, analgesia effects and the toleration oxygen deficit of the porslane [J]. *Chinese Journal of Hospital Pharmacy*, *22*.
17. XU Fang-qin, DU Xiao-bo, YAO Min-na. (2016). Protective Effects of Aqueous Extract from Portulacaoleracea L. on Ulcerative Colitis[J]. Progress in Modern Biomedicine, (32); 6239-6243.
18. Huang, Y., & Dong, L. (2011). Protective effect of purslane in a rat model of ulcerative colitis. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica*, *36*(19), 2727-2730.
19. LIU, L., ZHANG, H., & MA, W. J. (2011). Effects of Purslane Water Decoction on IL-8 IL-10 in Colon Tissue of Ulcerative Colitis in Mice [J]. *Journal of Liaoning University of Traditional Chinese Medicine*, *5*.
20. Dymicka-Piekarska, V., Matowicka-Karna, J., Gryko, M., Kemona-Chętnik, I., & Kemona, H. (2007). Relationship between soluble P-selectin and inflammatory factors (interleukin-6 and C-reactive protein) in colorectal cancer. *Thrombosis research*, *120*(4), 585-590.
21. Alonzi, T., Newton, I. P., Bryce, P. J., Di Carlo, E., Lattanzio, G., Tripodi, M., ... & Poli, V. (2004). Induced somatic inactivation of STAT3 in mice triggers the development of a fulminant form of enterocolitis. *Cytokine*, *26*(2), 45-56.
22. Gao, L., Liu, J., Tian, Y. J., & Shi, Y. J. (2006). Study on the Extraction and Purity Technology of Polysaccharide from *Portulaca oleracea* L. *Food Res. Dev*, *4*, 59-62.
23. FAN, Zhiqiang. (2017). Research Progress on Extraction Technology of *Portulaca oleracea* Polysaccharide[J]. Agricultural Science & Technology and Equipment, 8; 49-50.
24. Huang, C. (2014). Study on the Extraction of Polysaccharides from *Portulaca oleracea* L. by Ultrasound-assisted Extraction Method [J]. Journal of Jilin Agricultural Science and Technology University, *23*(3); 9-11.
25. Murano, M., Maemura, K., Hirata, I., Toshina, K., Nishikawa, T., Hamamoto, N., ... & Katsu, K. (2000). Therapeutic effect of intracolonically administered nuclear factor κB (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. *Clinical & Experimental Immunology*, *120*(1), 51-58.
26. CUI, Guoning, L.I.U., Xiping, ZENG, Q. (2018). Treatment Progress of UC by Shenling Baizhu Decoction [J]. Chinese Archives of Traditional Chinese Medicine, 2018, 2: 391-395.
27. Yanwei, F.U. (2021). Research Progress in the Treatment of Ulcerative Colitis with Chinese Herbal Decoction [J]. *Clinical Journal of Traditional Chinese Medicine, 33*(7);1402-1405.
28. Liang, P., Mao, L., Ma, Y., Ren, W., & Yang, S. (2021). A systematic review on Zhilong Huoxue Tongyu capsule in treating cardiovascular and cerebrovascular diseases: pharmacological actions, molecular mechanisms and clinical outcomes. *Journal of Ethnopharmacology*, 114234.