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番石榴多糖的制备及其改善溃疡性结肠炎作用探索

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Summary. *Ulcerative colitis (UC) is a chronic non-specific intestinal disease, which has the characteristics of long course and easy recurrence. In recent years, more and more attention has been paid to the role of plant polysaccharides in the prevention and treatment of ulcerative colitis. Therefore, in this study, pectinase and papain were added to the extraction of guava, and the compound enzyme (pectinase+papain) assisted extraction technology of guava polysaccharide was explored through single factor experiment to prepare guava polysaccharide. Further, the UC mouse model was established by dextran sodium sulfate (DSS), and the effect of guava fruit polysaccharide on UC improvement was investigated, so as to lay a theoretical and experimental foundation for the application of guava in UC prevention and treatment.*

Guava polysaccharide has been reported to have anti-tumor, anti-inflammatory, immune enhancement, anti-aging, blood sugar lowering and intestinal micro-ecology improvement effects. However, the improvement effect of guava polysaccharide on ulcerative colitis has not been reported. Enzymatic extraction of polysaccharides has the advantages of high extraction efficiency and simple post-treatment. Studies have reported that low molecular weight polysaccharides and oligosaccharides have certain advantages in the prevention and treatment of ulcerative colitis.

A large number of crude polysaccharides were extracted by the optimum process. 16.84 g crude polysaccharides were obtained from 102 g guava powder after fat removal, and 12.53 g guava polysaccharides were obtained after protein removal and dialysis. The total sugar content of guava polysaccharide was determined by phenol-sulfuric acid method with glucose as standard. With glucose concentration mg/mL as abscissa and absorbance value A at 490 nm as ordinate, draw the standard curve to get the regression linear equation: $y = 1.0467x – 0.0016$, $R2 = 0.9935$. With bovine serum albumin concentration mg/mL as abscissa and absorbance value A at 595 nm as ordinate, a standard curve was drawn, and the regression linear equation was $y = 0.3095x + 0.2155$, $R2 = 0.9974$. Finally, the total sugar content of the prepared guava polysaccharide is 86.09 %, and the average protein content is only 0.25 %, which indicates that the purity of the prepared guava polysaccharide is high.

DSS is a kind of heparin-like sulfated polysaccharide that can inhibit blood coagulation, enhance hypoxia of colonic mucosa, and aggravate intestinal bleeding. It has been proved in the literature that C57BL/6J mice are sensitive to DSS-induced colitis, and the pathological changes are similar to those of UC in human clinical histology. Therefore, in this paper, DSS-induced C57BL/6J mouse ulcerative colitis model was used to investigate the preventive and therapeutic effects of guava fruit polysaccharide on ulcerative colitis. The results showed that the stool of the blank group mice was normal and granular. Glossy fur, normal mobility, and steady increase in body weight. After drinking 2.5% DSS water for 2 days, the mice in the model group had diarrhea in different degrees, and their skin had no normal luster, which showed obvious decline in the ability to digest food, laziness, weakness and other pathological states. On the 5th day of treatment, compared with the normal control group, the weight of mice in DSS treatment groups decreased, especially in UC model group. The weight of mice in the high-dose and low-dose groups of guava polysaccharide was higher than that in the model group . In addition, the color, energy recovery and activity of mice in each dose group of guava polysaccharide were better than those in the model group. On the 5th and 6th day of treatment, compared with UC model mice, the symptoms of diarrhea and hematochezia in guava group were mild. The DAI score of guava polysaccharide in each dose group is significantly lower than that of the model group, indicating that guava polysaccharide has a good effect of preventing and treating UC. Although the middle dose group also showed improvement effect, the effect of improving body weight was not as obvious as that of the low and high dose groups.

UC can shorten the length of colon and damage the intestinal mucosal barrier . In order to further investigate the improvement effect of guava polysaccharide on UC, the effect of guava polysaccharide on

pathological tissues of mouse colon was further evaluated by naked eye observation, length measurement and tissue section. The colon length of the blank group mice is normal, and there is no obvious congestion, edema and ulceration observed by naked eyes. Compared with blank mice, UC mice in DSS model group have significantly shortened colon length, hyperemia and edema of colon mucosa, local erosion, bleeding and shallow inner wall, which involves chronic ulcer of colon mucosa of the whole mice. The colon length of mice treated with low, middle and high doses of guava polysaccharide was longer than that of DSS model group, and the symptoms of colon congestion, redness and swelling were relieved, and the inner wall ulcer was reduced, especially in the high and low dose groups of guava polysaccharide, which was better than the positive drug mesalazine.

HE staining analysis showed that the epithelial structure of colon mucosa of normal and control mice was intact, glands were distributed neatly, goblet cells were closely arranged, and there was no inflammatory infiltration. In DSS model group, colonic mucosa epithelium was severely damaged and deformed, with disordered structure, gland deformation shrinking or completely disappearing, crypt tissue atrophy or deformation, a large number of inflammatory cells infiltration in mucosa and its lower layer, and occasional crypt abscess formation. In the positive mesalazine group, goblet cells and crypts appeared, but there were still a few inflammatory cells in the conjunctival layer. Compared with the positive drug mesalazine group, guava polysaccharide group found that the colon structure was relatively complete, a large number of goblet cells appeared, and the glands were relatively complete, which was greatly improved compared with DSS model group, especially in the high dose group.

Ulcerative colitis, as a chronic and refractory bowel disease, lacks a radical cure clinically, so it is in urgent need of adjuvant therapy with high safety and little side effects. In this experiment, firstly, the extraction technology of guava polysaccharide was optimized by compound enzyme method. Through single factor method, it was determined that the extraction temperature was 70 ℃, the ratio of solid to liquid was 1: 50, papain was 500 U/g, and pectinase was 1100 U/g to obtain the highest yield of guava polysaccharide. Then, the UC mouse model was induced by DSS, and it was found that guava polysaccharide could alleviate the pathological state of UC mice at the dose of 50–250 mg/kg, and the effect of high dose guava polysaccharide was better than that of mesalazine. Although the experimental results reveal the potential of guava polysaccharide to improve ulcerative colitis, it also has some limitations. First of all, only two enzymes were selected in this experiment. The application of other enzymes, such as cellulase, in the extraction of guava polysaccharide deserves further discussion. In addition, this experiment only evaluated the effect of guava on improving ulcerative colitis at the whole animal level, and the specific molecular mechanism of its action needs further study. In addition, the guava polysaccharide used in this experiment is crude polysaccharide. Further separation and purification of guava polysaccharide, and further research on polysaccharide structure such as molecular weight analysis, monosaccharide composition, sugar chain connection sequence and position determination will help to better understand the function characteristics of guava polysaccharide and develop related products with clearer structure.

To sum up, guava crude polysaccharide has certain efficacy in preventing and treating ulcerative colitis. Guava is widely planted in South China and East China, and is rich in plant resources. Guava itself has the advantages of long fruit harvesting period, quick planting effect and strong comprehensive development and utilization, so it has a broad market prospect. The experimental results of this study will lay an experimental foundation for the optimization of the preparation technology of guava polysaccharide, and provide a scientific basis for the further development and utilization of guava and its polysaccharide components as functional foods and dietary nutritional supplements for adjuvant treatment of ulcerative colitis.