International Immunopharmacology 46 (2017) 163–169

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

The pharmacodynamic active components study of Tibetan medicine *Gentianopsis paludosa* on ulcerative colitis fibrosis

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ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 2 January 2017
Accepted 3 January 2017
Available online 11 March 2017

Keywords:
Tibetan medicine
Gentianopsis paludosa
Ulcerative colitis fibrosis
Active component

ABSTRACT

The current study focused on the pharmacodynamic activity components of *Gentianopsis paludosa* against ulcerative colitis (UC) fibrosis including symptoms of intestinal diarrhea and inflammatory. Trinitro-benzene-sulfonic acid induced UC model rats were gavaged with gradient polarity extracts respectively from ethanol-extract of *Gentianopsis paludosa*. Masson staining and qRT-PCR methods were respectively used to assess the degree of UC fibrosis and detect the mRNA expressions of collagen I, collagen III, a-smooth muscle actin (α-SMA) and E-cadherin in colon tissue. Separated by silica gel column chromatography, further screening was conducted until active components appeared. Infrared, nuclear magnetic resonance, mass spectroscopy and ultraviolet methods were applied to confirm active components' structures. The results indicated that the expression of collagen I, collagen III and α-SMA mRNA in the colon tissues of acetidin group rats was obviously depressed compared with control groups while E-cadherin displayed just opposite. Dyed in blue indicating UC fibrosis degree, the area of acetidin group was less than that other experimental groups. Four components: (1,8-Dihydroxy-3,7-Dimethoxyxanthones, 1-hydroxy-3,7,8-Trimethoxyxanthones, 1,7-Dihydroxy-3,8-Dimethoxyxanthones and 1-hydroxy-3,7-Dimethoxyxanthones), were obtained from acetidin group and all of which have a significant equivalence to *Gentianopsis paludosa* on the therapeutic effect of UC fibrosis. Our findings revealed the activity components for clinical application history of *Gentianopsis paludosa* and provided a preliminary foundation for further new drug research and exploitation.

1. Introduction

Gentianopsis paludosa (Hook.f.) Ma is an annual plant of *Gentianaceae gentianopsis* family. Tibetan people called it *jiadi*, *jihedou*, etc. [11]. It can be found in China, Nepal, India, Bhutan and Sikkim [10,12]. In China, it grows in the northwest region like province of Qinghai, Xizang, and south of Gansu with high altitude (2500–4500 m), meadow, damp marshes environment [24,26]. The whole herb is used for the treatment of conjunctivitis, hepatitis, nephritis, gastroenteritis, dyspepsia, fever, influenza and diarrhea in indigenous medicine [4,15]. Tibetan doctors used the plant to cure intestinal catarrh, dysentery especially for diarrhea [14,25]. To our knowledge, Wang, et al. [20] have reported anti-diarrhea and antibacterial activity of *Gentianopsis paludosa* but the corresponding active components are still unclear. The clinical symptoms of UC are diarrhea, abdominal pain, fever, bloody stool and other symptoms which are in line with the efficacy of *Gentianopsis paludosa*: anti-inflammatory, anti-diarrhea. Clinically, diarrhea is an important symptoms of UC fibrosis. Colonic fibrosis is a further serious stage of UC. Therefore, *Gentianopsis paludosa* often used in the treatment of UC. Previously, we have confirmed the role of *Gentianopsis paludosa* in the treatment of UC fibrosis [6–8]. Thus in this study, we studied the active components in order to support its application in the treatment of UC fibrosis and lay a foundation for further promising drug research.

UC fibrosis was distinctive pathology characteristics with a serious risk resulting in UC canceration. The current therapeutic tools were limited and recurrent intestinal inflammation seriously affect the quality of patients' life [18]. Recently, scholars believe UC fibrosis, was caused by many kinds of factors based on predisposing genes, such as cytokine, fibroblast and extracellular matrix interaction and these factors result in considerable col I, col III collagen deposited in bowel wall. There are only four types of collagen fiber molecules, in which collagen I (col I), collagen III (col III) are only the main synthesizers of fibroblasts. The expression of the two genes is significant for the formation of collagen fibers resulting in colonic fibrosis. Epithelial-mesenchymal transition (EMT) plays a crucial role during the process [2]. In the view of morphology, EMT was the process that enterocyte phenotype transform to interstitial cell thus gaining the shift ability. During the process, phenotype in enterocyte disappears and be replaced by considerable fibrosis interstitial cell classics marker like α-SMA, which resulting in formation

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http://dx.doi.org/10.1016/j.intimp.2017.01.001
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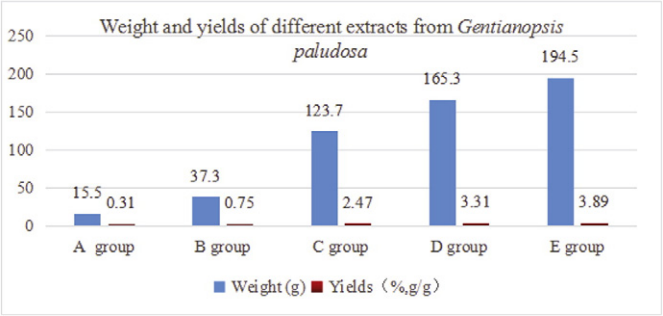

Fig. 1. Weight and yields of different extracts from Gentianopsis paludosa.

of UC fibrosis [17]. Compared with the distribution of β, γ-type of actin fiber in muscle cells and non-muscle cells, α-type SMA distributes in a variety of muscle cells, the more likely reflected in the process of colon tissue fibrosis. E-cad is an important cell-cell adhesion protein, which has been recognized as a metastasis suppressor of cancer cells [16]. The occurrence of tumor often cause intercellular adhesion decreased and E-cad expression declined. Hence, the present study was to choose col I, col III, α-SMA and E-cad as indicators to evaluate and define the effect of *Gentianopsis paludosa* treating UC fibrosis. Pathological staining (Masson staining), as a classic method to determine the degree of fibrosis, was taken as a supplementary method to assess the fibrosis in colon tissue.

2. Experimental

2.1. Plant

2.1.1. Plant collection and identification

Gentianopsis paludosa were collected from *YE Liguan* Town, Lintan County, *Gansu* Province, China, and identified by Prof. ChengYuan, *Gansu* Agricultural University. The voucher specimen (no. GSZY20140719001) was deposited in our herbarium.

2.1.2. The preparation and separation of ethanol extract of Gentianopsis paludosa

Gentianopsis paludosa powder (5000 g) was soaked in 95% ethanol (10000 mL) for 12 h, and reflux extracted 3 times. Filter the extraction and remove ethanol under low pressure to obtain extracta. Added distilled water (290 mL) to the extracta then sonicated for 10 min to make it fully suspended. Extracted the suspension with petroleum ether, chloroform, acetidin, n-butanol (suspl: solvent = 1:0.5, v/v) by turns until the solvent layer became colorless. Each part was concentrated at 40 °C, 0.08 MPa, after being dewatered by anhydrous Na_2SO_4. Then we got ligroin extractum, chloroform extractum, acetidin extractum, n-butanol extractum and water extractum. All the extractum were evaporated to dryness under thermostat-controlled water-bath at 45 °C and got them crashed, reserved at 4 °C temperature.

2.2. Reagents and apparatus

2.2.1. Reagents

Trinitro-benzene-sulfonic acid (TNBS, Sigma Company); sulfasalazine (SASP, three-dimensional Shanghai Pharmaceutical Co., Ltd.); TRIzol reagent (Invitrogen Co.); RT kit (Gene Company); RT-PCR kit (ABI company); PCR primers were synthesized according to the sequence in GenBank; Column chromatography on silica gel and high efficient silica G (Qingdao Marine Chemical Co., Ltd.); 95% ethanol, petroleum ether, chloroform, acetidin, n-butanol and other chemical reagents were analytical grade.

2.2.2. Apparatus

Waters600 HPLC (Waters Corporation USA); ZDM4000 mass spectrometer (Waters, USA); DRX-400 NMR Spectrometer (Burker, Germany); C1000RT-PCR instrument (BIO-RAD, USA); UV-3600 spectrophotometer (Shimadzu, Japan); VELOCITY18R high-speed refrigerated centrifuge (Dynamica, Australia); Burkersl-55 infrared spectrometer (Shimadzu, Japan).

2.3. Animals

2.3.1. Make UC fibrosis rats model

The process was conducted according to the earlier report [22]. After fasting diet but water 24 h later, the wistar rats were anesthetized by intraperitoneal injection of 2% aqueous solution of pentobarbital sodium, then inserted a 2 mm diameter polyethylene tube to rat anus 8 cm before injecting TNBS (150 mg/kg) alcohol solution (50%). Maintained superior position of anus for 3 min to ensure TNBS alcohol solution could not effuse from anus. Rats of normal group were coloclysised by isovolumic 0.9% NaCl solution and the remaining process kept the same to experimental group.

2.3.2. Group and treatment

The rats were randomly divided into 7 groups: petroleum ether (A group), chloroform (B group), acetidin (C group), n-butanol (D group), water (E group), model group, SASP positive group and another 10 healthy rats were selected randomly as normal group. The powder of each extract group was prepared for suspension respectively associated with 0.5% aqueous sodium CMC-Na. Refer to adult daily dose (0.36 g crude drug/kg), intragastric administration of each extract with the dose of 11.2; 27.0; 89.0; 119.2; 140.0 mg/kg for 28 days (7 days before and 21 days after making UC model). The model group and the normal group was lavaged with 0.9% isovolumic NaCl solution, while the positive group was lavaged with SASP suspension with the dose of 30.0 mg/kg.

2.3.3. Collect sample

Rats were killed by injecting Pentobarbital Sodium after 21 days of making UC model. End-piece colon at the length of 10 cm from anus was selected. Cut along the mesenterium longitudinally then flushed it with glacial saline. Before quickly stored at −80 °C liquid tank quickly, the segments obtained of colon tissue were dried by filter paper.

All experimental animals were overseen and approved by the Institutional Animal Care and Use Committee of *Gansu* University of Chinese Medicine before and during the experiment.

2.4. Methods

2.4.1. RT-PCR method

Homogenized the colon tissue (100 mg) in homogenizer under ice-cooling conditions and added cold saline to formulated 10% colon tissue homogenate. The process was conducted according to the earlier report [27]. RNA was extracted according to TRIzol kit instructions and cDNA were formed by reverse transcription. The total reaction volume was 20 μL. cDNA was conducted to fluorescent quantitation PCR. The design of primer based on Table 1. The total reaction system were: real time PCRMIX 15 μL; forward and reverse primers were each 0.5 μL; fluorescent probe (TaqMan) 0.5 μL; Taq DNA pclymerase 1 μL; ddH_2O 28 μL; cDNA template 5 μL, and the total volume was 50 μL. Reaction conditions: 50 °C 2 min; 95 °C 5 min; 95 °C 15 s; 60 °C 45 s, for 40 cycles. The RT-PCR data were normalized by the $2^{-\Delta\Delta Ct}$ algorithm. Calculated the Ct values using ABI Prism7500 SDS software.