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Portulacaoleraceal extract alleviates trinitrobenzene sulfonic acid-induced colitis in rats

Yun Huang[a,b], Jian Yin[c], Jian-Peng Gao[b], Yan Wang[a], Lei Dong[a,*], Ju-Hui Zhao[a,*]

[a] Department of Gastroenterology, Second Affiliated Hospital, Xi'an Jiaotong University, Shaanxi, China
[b] Department of Gastroenterology, Affiliated Yan'an Hospital of Kunming Medical University, Kunming, China
[c] Department of Gastroenterology, Xi'an Electric Power Central Hospital, Shaanxi, China

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ABSTRACT

Portulacaoleraceal (POL) has been widely used as an edible plant and a folk medicine in many countries, due to its several health benefits. This study examined the effects of POL on trinitrobenzene sulfonic acid (TNBS)-induced colitis via enema administration. Sixty male Sprague–Dawley rats were randomly divided into five groups: untreated, TNBS, TNBS + POL 10 g/kg, TNBS + POL 5 g/kg and TNBS + POL 2.5 g/kg groups. Rats were subjected to enema treatment once a day for 10 consecutive days with POL extract or distilled water after induction of TNBS. The changes of body weight, histological parameters, myeloperoxidase (MPO), superoxide dismutase (SOD), nitric oxide synthase activity (NOS), malondialdehyde (MDA) and nitric oxide (NO) levels in colon tissues were investigated. After POL extract treatment, body weights of rats significantly increased, macroscopic and microscopic damage scores reduced, MPO and NOS activity, as well as MDA and NO level significantly decreased, while SOD activity increased in a dose-dependent manner in the TNBS + POL groups compared with the TNBS group. Our results demonstrated that POL enema treatment attenuated pathologic changes of TNBS-induced colitis in rats through restoring colonic damage and reducing inflammatory response in the intestine. Thus, POL enema might be considered as a potential effective treatment for ulcerative colitis patients.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease affecting the colon, and its incidence is rising worldwide [1,2]. The typical clinical symptom of UC is bloody diarrhoea, urgency to evacuate and tenesmus. The clinical course is marked by alternating periods of exacerbation and remission, which may occur spontaneously or in response to treatment. Its aetiology and pathogenesis are very complex. UC is difficult to cure and frequently relapse. Recent studies have indicated that oxidative stress and inflammatory disorders are involved in the development of UC, thus these pathways may probably represent efficacious therapeutic targets for developing new drugs [3,4].

Portulacaoleraceal (POL), an herbaceous succulent annual plant belonging to the Portulacaceae family, is widely distributed in warm climate. It has been widely used as an edible plant and a folk medicine in many countries [5]. Flavonoids, alkaloids, fatty acids, terpenoids, and polysaccharides are the major bioactive components in POL[6]. POL has been attracted extensive attention because of its many pharmacological activities, such as antibacterial [7], antiulcerogenic [8], anti-inflammatory [9], antioxidant [10], and wound-healing [11] properties.

Studies have reported that POL decreases high fat diet-elicited oxidative damage through modulating blood and liver antioxidant enzyme activities [12]. POL also inhibits tumor necrosis factor-α-induced production of intracellular reactive oxygen species (ROS) in a dose-dependent manner [13]. POL has a high potential to be used as a pharmacological agent in UC treatment. However, little is known regarding the effect of POL on the ulcerative colitis. The purpose of the present study was to evaluate whether POL treatment attenuates UC inflammatory response via modulating antioxidant enzyme activities and ROS level.

2. Materials and methods

2.1. Materials and chemicals

POL extract powder (batch number MC-071010) was purchased from Hongda Plant Chemical Co. Ltd. (Xi'an, Shaanxi, China). POL

* Corresponding authors at: Department of Gastroenterology, Second Affiliated Hospital, Xi'an Jiaotong University, Shaanxi, China.
E-mail addresses: huangyun9@126.com (Y. Huang), indiagpp@163.com (J. Yin), gaojianpengkm@163.com (J.-P. Gao), miss.wangyan@gmail.com (Y. Wang), dong556@126.com (L. Dong), juhui801010@mail.xjtu.edu.cn (J.-H. Zhao).

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extract powder is a water extract from POL whole grass (1:10). The composition of POL extract powder is a mixture including flavonoids, alkaloids, fatty acids, terpenoids, polysaccharides, vitamins, sterols, proteins, and minerals. Trinitrobenzene sulfonic acid (TNBS) 5% (W/V) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Myeloperoxidase (MPO), superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and nitric oxide synthase activity (NOS) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

2.2. Animals

Sixty male Sprague–Dawley rats (6–7 weeks old, weighing 180–200 g) were obtained from the Laboratory Animal Center of Xi'an Jiaotong University. Rats were maintained with food and water available ad libitumin in an air-conditioned room at a temperature of 23 ± 2 °C and a relative humidity of 60 ± 5%, under a 12/12 h light/dark cycle. All experiments were conducted following the institutional ethical guidelines. All efforts were made to minimize the number of animals and their suffering.

2.3. Colitis induction and experimental design

Rats were randomly divided into five groups (n = 12/group): untreated, TNBS, TNBS + POL (2.5, 5, 10 g/kg). TNBS-induced colitis was performed as previously described [14]. Rats were lightly anesthetized with ether and a plastic catheter (2 mm diameter) was inserted into the colon for a length of 8 cm through the anal sphincter. Colitis was induced by the administration of 1 ml TNBS (TNBS 25 mg dissolved in 1 ml of 50% ethanol) through the plastic catheter. After TNBS administration, the rats were kept in a head-down position for 60 s to prevent expulsion of TNBS. The untreated group received 1 ml of physiological saline using the same procedure. Rats had free access to water during the experiment. Rats in the TNBS + POL groups were administrated POL enema once a day for 10 consecutive days after 3 days of TNBS administration. POL extract was dissolved in distilled water. Rats in the untreated and TNBS groups received an equal volume of distilled water by enema. During the experiments, total 12 rats died including 4 in the TNBS group, 3 in the TNBS + POL 10 g/kg, 2 in TNBS + POL 5 g/kg and 3 in TNBS + POL 2.5 g/kg groups.

2.4. Colitis assessment and sample collection

All rats were weighed daily during the entire experimental period. After a fasting period of 18–24 h with free access to 5% glucose solution, they were sacrificed by cervical dislocation at day 14. Entire colon was excised, longitudinally opened and washed with cold saline to remove faecal residue. The colon was immediately placed on ice to score colonic mucosa damage according to previously established macroscopic damage grade criteria (Table 1) [15]. One small section ($0.5 \times 0.5\ cm^2$) from a clear colon lesion was fixed in 4% paraformaldehyde phosphate buffer solution for 24 h. The other section from a clear colon lesion was frozen in liquid nitrogen and stored at −80 °C.

Table 1
Macroscopic damage grade criteria in trinitrobenzene sulfonic acid-induced colitis in rat.

Score	Parameter
0	No damage
1	Hyperaemia without ulcers
2	Hyperaemia and bowel wall thickening without ulcer
3	One site of ulceration without bowel wall thickening
4	Two or more sites of ulceration or inflammation
5	0.5 cm of inflammation and major damage
6–10	1 cm of major damage. The score was increased by 1 for every 0.5 cm damage found to a maximum of 10.

Table 2
Microscopic damage grade criteria in trinitrobenzene sulfonic acid-induced colitis in rat.

Score	Parameter
0	Normal colonic tissue
1	Inflammation or focal ulceration limited to the mucosa
2	Focal or extensive ulceration and inflammation limited to the mucosa and submucosa
3	Focal or extensive ulceration and inflammation limited to muscularis propria
4	Focal or extensive ulceration and inflammation limited to serosa
5	Extensive ulceration and transmural inflammation with serosa involvement

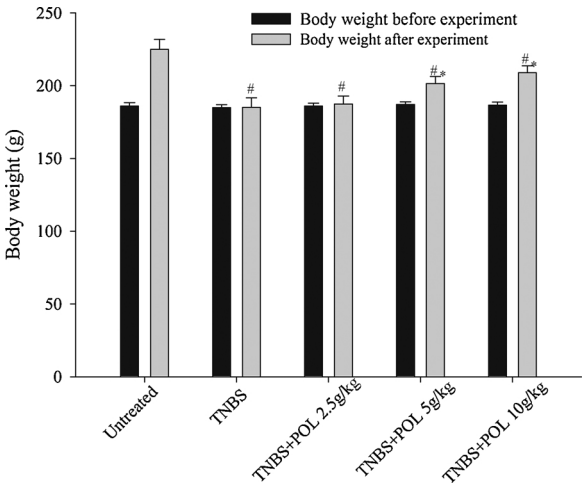

Fig. 1. Effect of POL on body weight change in TNBS -induced colitis rats. # $p < 0.01$ vs. untreated group. * $p < 0.01$ vs. TNBS group. TNBS, trinitrobenzene sulfonic acid; POL, portulacaoleraceal.

2.5. Histological examination

The paraformaldehyde section was routinely dehydrated, paraffin embedded, sliced, dewaxed, hydrated and HE stained. Two experienced pathologists observed and evaluated the slides using a double-blind method. The colonic microscopic damage score was evaluated according to previously established criteria (Table 2) [16].

2.6. MPO, SOD, NOS activity, MDA and NO level measurement in colon tissues

One gram of colon samples stored at −80 °C was homogenized in 9 ml of cold physiological saline. An aliquot of homogenate was used for measuring MPO activity. The other homogenate aliquot was centrifuged at 3000 *g* at 4 °C for 10 min, then the supernatant was transferred into several new tubes, and frozen in −80 °C to measure SOD, MDA, NO and NOS contents using the corresponding assay kits according to the provider's instructions. MPO activity was expressed as U/g wet colon tissue and defined as the quantity of enzyme degrading 1 μmol of peroxide per minute at 37 °C. MDA level was expressed as nmol MDA per milligram of protein in wet tissues (nmol/mg). SOD and NOS activities were expressed as units per milligram of protein in wet tissues (U/mg). NO level was expressed as μmol NO per gram of protein in wet tissues (μmol/g).

2.7. Statistical analysis

Results are presented as mean ± standard deviation. Statistical