Journal of Ethnopharmacology 162 (2015) 7–13

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Red Ginseng and *Semen Coicis* can improve the structure of gut microbiota and relieve the symptoms of ulcerative colitis

Mingzhang Guo [a,1], Shuo Ding [a,b,1], Changhui Zhao [c], Xinxi Gu [b], Xiaoyun He [a], Kunlun Huang [a], Yunbo Luo [a], Zhihong Liang [a], Hongtao Tian [b,*], Wentao Xu [a,**]

[a] College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China
[b] College of Food Science and Technology, Agricultural University of Hebei, Baoding 071001, PR China
[c] Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742, USA

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
21 November 2014
Accepted 20 December 2014
Available online 30 December 2014

Keywords:
Chinese herbs
Gut microbiota
Ulcerative colitis

ABSTRACT

Ethnopharmacological relevance: Many Chinese herbs are traditionally used as medicine to improve the functions of gastrointestinal tract. Some of these herbs are also promising agents for the improvement of the gut microbiota and the treatment of ulcerative colitis.

Materials and methods: By screening seven traditional Chinese herbs, we found that *Red Ginseng* and *Semen Coicis* were the most effective in promoting the growth of probiotics including *Lactobacillus* and *Bifidobacterium in vitro*. We then evaluated the effects of *Red Ginseng* and *Semen Coicis* on the growth of the bacterial pathogens *(Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.) in vitro*. In *in vivo* experiment, we gavage administrated trinitro–benzene–sulfonic acid induced ulcerative colitis (UC) rats with *Red Ginseng* and *Semen Coicis* extracts. After two weeks treatment, we analyzed the structure of the gut microbiota and examined the UC symptoms by employing qPCR and animal pathology detection techniques.

Results: Both *Red Ginseng* and *Semen Coicis* promoted the growth of probiotics – *Bifidobacterium* and *Lactobacillus in vitro*. *Red Ginseng* also inhibited the growth of some pathogen strains. *In vivo, Red Ginseng* and *Semen Coicis* improved the structure of gut microbiota and relieved the symptoms of ulcerative colitis *in vivo*. Compared with *Semen Coicis*, *Red Ginseng* was more effective in relieving the symptoms of ulcerative colitis.

Conclusions: Red Ginseng could promote the growth of probiotic bacteria in vitro. *Red Ginseng* and, to a lesser extent *Semen Coicis*, gave positive results in an experimental in vivo model for ulcerative colitis.

1. Introduction

Gut microbiota is a dynamic “organ” of critical importance for human health. In healthy condition, the symbiotic microorganisms in intestinal tract participate in the normal nutrient metabolism and immunity regulation of the host (Ahern et al., 2014; Flint et al., 2012). However, disorder of the microbiota's composition is implicated in a variety of diseases (Barnich and Darfeuille-Michaud, 2007; Karlsson et al., 2012; Lozupone et al., 2013; Qin et al., 2012). Although it is difficult to evaluate the causal effect of the microbiota disorder on these diseases in most cases, it is agreed that modification of the microbiota structure by increasing the amount of probiotics or inhibiting the growth of potential pathogens is beneficial for the maintenance of host health and the remission intestinal diseases, such as ulcerative colitis (UC) (Bellavia et al., 2013).

Antibiotics are frequently used to treat intestinal diseases, including UC by inhibiting the growth of pathogens (Swidsinski et al., 2008). The central concern of the use of antibiotics is the increased abundance of antibiotic resistant genes that are capable of drifting to the pathogens (Martinez, 2008). Moreover, antibiotics with broad-spectrum antibacterial effect may also inhibit the native gut probiotics that lack resistant genes in their genomes.

A great variety of Chinese herbs have been used as medicines to treat intestinal diseases for thousands of years in Asia. The beneficial effect of these herbs is, at least partly, achieved by modulation of gut microbiota. Different from the broad-spectrum antibacterial effects of antibiotics, some herbs have promoting or inhibiting effects on specific gut bacterial species. Berberine, the major pharmacological component of *Rhizoma coptidis* was found to induce structural changes of gut microbiota by enriching specific bacterial species, including *Blautia* and *Allobaculum*, two putative short-chain fatty acid-producing bacteria

* Correspondence to: No. 2596, Kaile South Avenue, Baoding, Hebei Province 071001, PR China. Tel./fax: 86 312 7528 423.
** Correspondence to: No. 17, Qinghua East Road, Haidian District, Beijing 100083, PR China. Tel./fax: 86 10 6273 7786.
E-mail addresses: tht631022@163.com (H. Tian), xuwentao@cau.edu.cn (W. Xu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jep.2014.12.029
0378-8741/

(Han et al., 2011; Zhang et al., 2012). Another example is the use of *Herba ephedrae*. The anti-obesity effect of *Herba ephedrae* was indicated to achieve by modulation of gut microbiota (Kim et al., 2014). These pioneering researches indicate that Chinese herbs are promising sources to find gut microbiota regulation materials.

In the present study, seven traditional Chinese herbs, including *Semen Coicis*, *Red Ginseng*, *Radix Glycyrrhizae*, *Rhizoma Dioscoreae*, *Fructus Amomi*, *Rhizoma Atractylodis Macrocephalae*, and *Radix Aucklandiae*, were screened for their potential to promote the growth of probiotics. According to the Compendium Of Materia Medica (Bencao Gangmu), a world famous traditional Chinese medicine work included in UNESCO's Memory of the World Register, these herbs could either improve the functions of gastrointestinal tract or treat the symptoms of diarrhea or dysentery (refer to "Pi Wei", "Xie Xie", and "Li" sections). After *in vitro* screen, the selected *Red Ginseng* (RG) and *Semen Coicis* (SC) were further evaluated in the treatment of experimental UC in rats. The results showed *Red Ginseng* promoted the growth of probiotic bacteria in vitro, and *Red Ginseng* and to a lesser extent *Semen Coicis* gave positive results in an experimental in vivo model for ulcerative colitis.

2. Materials and methods

2.1. Chinese herbs, herb-containing medium and bacterial strains

Seven Chinese herbs were purchased from Pukang Big Pharmacy (Baoding, Hebei province, China), including: (1) *Semen Coicis*, dried matured kernel of *Coix lacryma-jobi* L. var. mayuen (Roman.) Stapf, (2) *Red Ginseng*, dried root from steamed *Panax ginseng* C. A. Mey., (3) *Radix Glycyrrhizae*, dried root or rhizome of *Glycyrrhiza uralensis* Fisch., (4) *Rhizoma Dioscoreae*, dried rhizome of *Dioscorea opposita* Thunb., (5) *Fructus Amomi*, dried matured fruit of *Amomum villosum* Lour., (6) *Rhizoma Atractylodis Macrocephalae*, dried rhizome of *Atractylodes macrocephala* Koidz., (7) *Radix Aucklandiae*, dried root of *Aucklandia lappa* Decne. 100 g of each herb was steeped in 200 ml distilled water overnight. Then they were incubated in water bath at 95 °C for 3 h and filtered. The process was repeated once. The filtrate was concentrated to 100 ml by heating. The procedure above mimics the process of traditional decoction of Chinese herbs. The final solution was defined as 1 g/ml or 100% concentration. The herb solution was diluted with the culture medium (basic broth or Luria-Bertani broth) to 6.25%, 12.5%, 25%, and 50% herb-containing medium (v/v). Basic broth (beef extract 0.3 g, glucose 1 g, peptone 1 g, NaCl 0.5 g, cysteine hydrochloride 0.04 g, distilled water 100 ml) was used for probiotics cultivation, while Luria-Bertani (LB) broth was used for potential pathogens cultivation.

Bifidobacterium animalis, *Bifidobacterium longum* and *Lactobacillus rhamnosum* (provided by Laboratory of Fermentation Engineering, Agricultural University of Hebei, *Bifidobacterium longum* was isolated from the gut microbiota of healthy people, identified and numbered as Blm, *Bifidobacterium animalis* and *Lactobaccillus rhamnosum* were isolated from fermentation dairy, identified and numbered as 05-161 and 05-281 respectively) were used as the representatives of the probiotics. *Escherichia coli* BL21, *Staphylococcus aureus* ATCC 25923, *Salmonella spp.* CGMCC 1.0090 (provided by Laboratory of Food Safety and Molecular Biology, China Agricultural University) were used as the representatives of the potential pathogens.

2.2. Screening of herbs in vitro

The growth curves of three strains of probiotics in basic broth were obtained using Hungate technique (Hungate, 1969). *Bifidobacterium animalis*, *Bifidobacterium longum*, and *Lactobacillus rhamnosum* reached the end of logarithmic growth at 16 h, 16 h, and 12 h respectively. 12.5% and 25% of each of herb-containing basic broth were inoculated with 1% (v/v) activated probiotic strains (10^7 CFU/ml), and cultured under anaerobic condition at 37 °C. The OD_{600} of probiotics cultures was measured at the end of the logarithmic growth and 2 h before and after it with the broth without any inoculation as control. Compared with basic broth (without any herb) inoculated with the same amount of strains, the herbs that had the higher OD_{600} than the control are regarded as potential probiotics-promoting herbs. 6.25%, 12.5%, 25%, and 50% herb-containing basic broth of *Red Ginseng* and *Semen Coicis* were inoculated with 1% (v/v) activated probiotic strains (10^7 CFU/ml) and the viable counts were measured by the Hungate technique at the end of the logarithmic growth.

The growth curves of three strains of potential pathogens in LB broth were obtained by measurements of OD_{600}. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.* reached to the end of their logarithmic growth at 12 h, 14 h, and 16 h respectively after 200 r/min shaking cultivation at 37 °C. 6.25%, 12.5%, 25% herb-containing LB broth of *Red Ginseng* and *Semen Coicis* and LB broth without herb were inoculated with 1% activated pathogenic strains. The OD_{600} values of the cultures were measured at the end of logarithmic growth with the broth without any inoculation as control. Compared with LB broth without any herb, the pathogen inhibiting effects of *Red Ginseng* and *Semen Coicis* were evaluated.

2.3. Animal experiments

Male Wistar rats (7 weeks old) were purchased from Vital River, Beijing, China. All rats were housed in stainless steel cages with *ad libitum* access to the filtered tap water and the chow (Keaoxieli, Beijing, China) in an specific pathogen-free animal room at The Supervision and Testing Center for GMO Food Safety, Ministry of Agriculture (Beijing, China) with the license number SYXK (Beijing) 2010-0036. The animal room was maintained at a temperature of 22 ± 2 °C and a relative humidity of 40–70%. The room was artificially illuminated (fluorescent light) with a 12 h light/dark cycle. The air exchange rate was 15 times/h. The protocol was approved by the Animal Ethics Committee of China Agricultural University, Beijing (No. 130181).

The rats were randomly divided into three groups, i.e. model control (MC) group, *Red Ginseng* (RG) group or *Semen Coicis* (SC) group ($n=8$ per group). After a week of acclimatization, all the rats were treated with 100 mg/kg trinitro-benzene-sulfonic acid (TNBS, Sigma-Aldrich, St. Louis, MO, USA, dissolved in 50% ethanol) by enema to induce ulcerative colitis (Ha et al., 2012; Sanchez-Fidalgo et al., 2007). Rats of RG group and SC group were given the 1 g/ml herb solution at 4 ml/kg body weight by gavage. Rats of MC group were given the sterile saline solution at the same dose. The body weight, food intake and fecal characteristics of each rat were recorded to calculate the disease activity index (DAI) (Dharmani et al., 2011; Nagib et al., 2013). The standard scale for DAI evaluation was shown in Table 1.

After 2 weeks, the rats (fasting for 8 h) were anesthetized by chloral hydrate (6% w/v, 5 ml/kg), decapitated and dissected. Blood routine tests were performed. The length, weight, and mucosal damages of the colon of each rat were recorded rapidly, and then the colons were stored in formalin or frozen as soon as possible for further analysis. The colon index (the weight of colon/the length of colon) was calculated. Histopathological examinations were performed to examine the pathological lesions inside the colons. The malondiadehyde content (MDA) and myeloperoxidase activity (MPO) of colon tissues were measured to evaluate the severity of UC using the thiobarbituric acid method (Ou et al., 2007) and the hydrogen peroxide method (Massa et al., 2004) respectively. Other major visceral organs were also weighted and the organ coefficients (weight of organ/body weight) were calculated accordingly.