International Journal of Biological Macromolecules 66 (2014) 81–85

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Antioxidant Chinese yam polysaccharides and its pro-proliferative effect on endometrial epithelial cells

Ying Ju[a,1], Yan Xue[b,1], Jianlei Huang[a], Qingzhi Zhai[c], Xiao-hong Wang[a,*]

[a] Department of Obstetrics and Gynecology, Tangdu Hospital, Fourth Military Medical University, Xi'an 710032, China
[b] Department of Preventive and Health Care, Shanxi People's hospital, Taiyuan 30053, China
[c] Department of Obstetrics and Gynecology, Chinese PLA General Hospital, Beijing 100853, China

ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form 25 January 2014
Accepted 29 January 2014
Available online 12 February 2014

Keywords:
Chinese yam polysaccharide
Pro-proliferative
Antioxidant
Endometrial epithelial cells
Female infertility
Female reproduction

ABSTRACT

Chinese yam polysaccharide (CYP) was successfully isolated from the rhizoma of *Dioscorea opposita* and its chemical characteristics were determined. Antioxidant and pro-proliferative activity of CYP on human endometrial epithelial cells in vitro were investigated. Role of CYP on cell proliferation was also evaluated by examining pro-apoptotic and anti-apoptotic proteins Bax and Bcl-2 by Western blot analysis. Chemical composition analysis indicated that CYP was mainly composed of mannose, glucose, galactose and glucuronic acid in the ratio of 0.5:1.2:0.3:0.3. In vitro, CYP exhibited a potent scavenging activity on the DPPH radical, hydroxyl radical and superoxide radical. Furthermore, CYP apparently promoted the proliferation of human endometrial epithelial cells, especially beyond the concentration of 100 μg/ml after 36 h exposure. Western blot revealed that the anti-apoptotic protein Bcl-2 was upregulated after endometrial epithelial cells were treated with CYP, while the protein level of Bax was attenuated, thus leading to the downregulation of Bax/Bcl-2 ratio. Our findings provide the first evidence that CYP may prove to be a potential candidate of the natural antioxidants as a therapeutic agent for female infertility.

1. Introduction

Over the past two decades, the reported prevalence of infertility has increased markedly [1]. It is estimated that fifty percent of cases may be a result of female problems, including ovulatory disorders, poor egg quality, fallopian tube damage, endometriosis hydrosalpinges (dilated fallopian tubes), polycystic ovarian syndrome (PCOS), fetal malformations and potentially unexplained subfertility [2,3]. Free radicals and oxidative stress have been implicated in male and female infertility [4,5]. To overcome these fertility problems, many subfertile women undergoing fertility treatment take antioxidant dietary supplements in an attempt to improve their fertility [6]. Antioxidant therapy has been implicated to be effective in reducing the oxidative stress brought on by these conditions. Considering the advantages of lower side effects of natural antioxidants than those of synthetic antioxidants, plants or their extracts have been extensively explored in animals or human beings for potential therapy toward female subfertility.

Assisted reproduction technologies (ART) have provided considerable insight into the human reproductive processes. These include ovulation stimulation, intrauterine insemination, in vitro fertilization (IVF) and intracytoplasmic sperminjection (ICSI) [7]. The limiting factor in achieving pregnancy for most couples is implantation. During this process, endometrial receptivity is one of the key factors determining the likelihood of success in ART treatments [8]. It is widely accepted that a thin endometrial stripe on transvaginal ultrasound scan is associated with a poor pregnancy rate [9–11]. Furthermore, some authors have found that increased endometrial thickness would benefit implantation or pregnancy rates in patient suffered from AST [12,13]. The endometrium has the distinctive ability to undergo physiologic angiogenesis in order to facilitate implantation, as well as to regenerate an entirely new functional layer following each menses [14]. On the contrary, abnormal endometrial function remains a significant cause of implantation failure, recurrent pregnancy loss, and other pathologies which lead to female infertility [15]. Although the mechanisms which allow for it remain poorly understood, this remarkable regenerative capacity is essential for successful human reproduction. Therefore the searching for a novel natural antioxidant with little toxicity and pro-proliferative effect on endometrial epithelial cells would be highly urgent.

In recent years, plant derived nonstarchy polysaccharides have emerged as an important class of bioactive natural products. A wide range of polysaccharides have been demonstrated to play an important role as free radical scavengers in the prevention of oxidative damage in living organism and can be explored as novel

* Corresponding author. Tel.: +86 029 84777691; fax: +86 029 84777691.
E-mail address: xiaohongwang_fmmu@tom.com (X.-H. Wang).
[1] Ying Ju and Yan Xue contributed equally to this work.

http://dx.doi.org/10.1016/j.ijbiomac.2014.01.070
0141-8130/

potent antioxidant [16–18]. Rhizoma of *Dioscorea opposita* Thunb. (Chinese yam) is not only a common food in China regarded as a tonic, but also has been also used as traditional Chinese medicine (TCM) more than 2000 years for the treatment of diabetes, diarrhea, asthma and other ailments [19]. Chinese yam polysaccharide (CYP) is the main functional components in Chinese yam and has been proved by modern medicine that CYP are efficacious for anti-tumor, decreasing blood glucose, and modulating immune function [20–23]. However no document regarding the antioxidant and pro-proliferative effect of CYP on endometrial epithelial cells is available. Therefore, the purpose of the present study is to prepare the polysaccharides from Chinese yam and evaluated their antioxidant activities in vitro and pro-proliferative effect on human endometrial epithelial cells.

2. Materials and methods

2.1. Materials and chemicals

Rhizoma of *D. opposita* was purchased from An Guo Herbal Market (Hebei, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), ethylene diamine tetra-acetic acid (EDTA)–Fe, dihydromicotineamidadenine dinucleotide (NADH), Tris–HCl buffer, standard monosaccharides, trifluoroacetic acid (TFA), D-glucose, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in this study were of the highest quality available from commercial vendors.

2.2. Preparation of CYP

The air dried and crushed rhizoma of *D. opposite* (250 g) were first exhaustively extracted with methanol–chloroform (8:1, v/v) for 24 h under reflux to remove the interfering components, such as monosaccharide, disaccharide, oligosaccharide and polyphenol in the samples at 80 °C. After filtration, the residue was extracted with 2 L of bidistilled water at 100 °C for 3 times and 3 h for each time. The extract solution was combined, and then centrifuged at 3000 × *g* for 10 min, followed by passing through a 0.45 μm pore size filter to separate the supernatant and the residue. The associated proteins in the extracts were removed using the Sevag method [24]. After exhaustive dialysis with water for 48 h, the concentrated dialyzate was precipitated with four volumes of ethanol at 4 °C for 24 h to precipitate polysaccharides. Finally, the precipitate was washed with absolute ethanol, acetone and ether, respectively, giving CYP.

2.3. Chemical composition of CYP

Total carbohydrate content of the polysaccharide was determined by phenol–sulfuric acid colorimetric method [25]. Protein content was quantified according to the Bradford's method [26]. In addition, total uronic acid content in the polysaccharides was measured by m-hydroxydiphenyl method [27].

2.4. Monosaccharide composition analysis

Gas chromatography (GC) was used for identification and quantification of monosaccharides in CYP. The polysaccharide (10 mg) was hydrolyzed with 10 ml of 2 M TFA at 120 °C for 6 h [28], and the excess acid was completely removed by co-distillation with ethanol. Then the hydrolyzed product was reduced with KBH_4 (30 mg), followed by neutralization with dilute acetic acid and evaporated at 45 °C after adding 1 mg myo-inositol and 0.1 M Na_2CO_3 (1 ml) at 30 °C with stirring for 45 min. The residue was concentrated by adding methanol. Finally the reduced products (alditols) were added with 1:1 pyridine–propylamine at 55 °C with stirring for 30 min, and acetylated with 1:1 pyridine–acetic anhydride in a boiling water bath for 1 h. After cooling to room temperature, the acetylated derivatives were loaded into a DB-1 capillary gas chromatography (GC) column (30 m × 0.25 mm × 0.25 μm) equipped with a flame-ionization detector (FID) on a Shimadzu GC-9A instrument (Shimadzu, Japan), using inositol as the internal standard. The column temperature was programmed from 130 to 180 °C at 5 °C/min, kept at 180 °C for 2 min, and increased to 220 °C at a rate of 5 °C/min, and finally holding for 3 min at 220 °C. The flow rate of N_2, H_2 and air were 20 mL/min, 30 mL/min and 400 mL/min, respectively. The temperature of detector and inlet were 280 °C and 250 °C, respectively. Standard monosaccharides (rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose) were used as references.

2.5. IR spectroscopy

The IR spectrum of the polysaccharide (~0.5 mg) was determined using a Fourier transform infrared spectrophotometer (FTIR, Bruker, Germany) as KBr pellets at room temperature in a range of 400–4000 cm^{-1} [29].

2.6. Antioxidant activities of CYP

2.6.1. DPPH radical scavenging activity

The scavenging activity of CYP on the DPPH free radical was assayed using the method in the literature [30], with slight modification. Briefly, the sample solution (1 ml) with variable concentrations (25–400 μg/ml) was added to tube containing 1 ml of DPPH solution (0.2 mM in ethanol). This reaction mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. Then the absorbance was measured at 517 nm after 30 min on a spectrophotometer (U-2000 HITACHI, Japan) [31]. The scavenging ability of CYP on the DPPH radicals was calculated according to the following equation: scavenging effect (%) = $[1 - A_1/A_0] \times 100$, where A_0 is the absorbance of DPPH solution without the tested samples and A_1 is the absorbance of the tested samples with DPPH solution.

2.6.2. Hydroxyl radical scavenging activity

The scavenging activity of CYP on the hydroxyl radical was assayed by the method of Smirnoff and Cumbes [32], with slight modification. Briefly, the sample solution (1 ml) with variable concentrations (25–400 μg/ml) was added to 2 ml of phosphate buffer (100 mM, pH 7.4) containing EDTA-Fe (2 mM), ascorbic acid (0.1 mM), and H_2O_2 (1 mM) for 60 min at 37 °C. The hydroxyl radical was detected by monitoring absorbance at 520 nm. The scavenging ability of CYP on the hydroxyl radical was calculated according to the following equation: scavenging effect (%) = $[1 - A_1/A_0] \times 100$, where A_0 is the absorbance of the control (deionized water, instead of sample) and A_1 is the absorbance of the sample mixed with reaction solution.

2.6.3. Superoxide radical scavenging activity

The scavenging activity of CYP on the superoxide radical was assayed by modified methods reported by Robak and Gryglewski [33]. Briefly, 1 ml of different concentration of sample (25–400 μg/ml) was mixed with 1 ml of 0.1 M phosphate buffer (pH 7.4) containing 150 μM NBT, 60 μM PMS and 470 μM NADH. The reaction mixture was shaken thoroughly and then incubated at room temperature for 5 min, and the absorbance at 560 nm was measured. The scavenging ability of CYP on the superoxide radical was calculated according to the following equation: scavenging effect (%) = $[1 - A_1/A_0] \times 100$, where A_0 is the absorbance of control