J Ginseng Res xxx (2016) 1–11

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

The beneficial effect of ginsenosides extracted by pulsed electric field against hydrogen peroxide-induced oxidative stress in HEK-293 cells

Q9 Di Liu, Ting Zhang, Zhifei Chen, Ying Wang, Shuang Ma, Jiyun Liu, Jingbo Liu*

Laboratory of Nutrition and Functional Food, College of Food Science and Engineering, Jilin University, Changchun, China

ARTICLE INFO

Article history:
Received 16 September 2015
Received in Revised form
30 January 2016
Accepted 26 February 2016
Available online xxx

Keywords:
antioxidant activities
ginsenosides
oxidative stress
Panax ginseng
pulsed electric field

ABSTRACT

Background: Ginsenosides are the main pharmacological components of *Panax ginseng* root, which are thought to be primarily responsible for the suppressing effect on oxidative stress.
Methods: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and oxygen radical absorption capacity were applied to evaluate the antioxidant activities of the ginsenosides. Human embryonic kidney 293 (HEK-293) cells were incubated with ginsenosides extracted by pulsed electric field (PEF) and solvent cold soak extraction (SCSE) for 24 h and then the injury was induced by 40μM H_2O_2. The cell viability and surface morphology of HEK-293 cells were studied using MTS assay and scanning electron microscopy, respectively. Dichloro-dihydro-fluorescein diacetate fluorescent probe assay was used to measure the level of intracellular reactive oxygen species. The intracellular antioxidant activities of ginsenosides were evaluated by cellular antioxidant activity assay in HepG2 cells.
Results: The PEF extracts displayed the higher 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and stronger oxygen radical absorption capacity (with an oxygen radical absorption capacity value of 14.48 ± 4.04μM TE per μg/mL). The HEK-293 cell model also suggested that the protective effect of PEF extracts was dose-dependently greater than SCSE extracts. Dichloro-dihydro-fluorescein diacetate assay further proved that PEF extracts are more active (8% higher than SCSE extracts) in reducing intracellular reactive oxygen species accumulation. In addition, scanning electron microscopy images showed that the HEK-293 cells, which were treated with PEF extracts, maintained more intact surface morphology. Cellular antioxidant activity values indicated that ginsenosides extracted by PEF had stronger cellular antioxidant activity than SCSE ginsenosides extracts.
Conclusion: The present study demonstrated the antioxidative effect of ginsenosides extracted by PEF *in vitro*. Furthermore, rather than SCSE, PEF may be more useful as an alternative extraction technique for the extraction of ginsenosides with enhanced antioxidant activity.

1. Introduction

Reactive oxygen species (ROS) have been implicated with cell oxidative stress injury, which result in disorders of physiological functions of DNA, proteins, lipids, and other macromolecules, and subsequently many diseases. When the maintenance of redox homeostasis is overwhelmed, exogenous antioxidants play a significant role in the body's redox homeostasis system. ROS were released and cyclin D1 was degraded by ubiquitin when human embryonic kidney 293 (HEK-293) cells were exposed to excessive H_2O_2, which was found to contribute to the induction of the cell cycle arrest in the G2 phase [1]. Exogenous extracts of antioxidants from dietary sources could be extremely useful in suppressing the accumulation of oxidative stress injury [2].

Panax ginseng as "the king of herbs" has been used as a Chinese traditional medicine for thousands of years in East Asia, known for its various beneficial effects on cardiovascular systems, central nervous, endocrine systems, and on sexual function [3]. Ginsenosides have been regarded as the main active ingredients of *P. ginseng*, and are used as a marker for assessing the quality of ginseng. There are many reports dealing with ginsenosides' pharmacological effects on oxidative damage. Lu et al [4] have

* Corresponding author. Laboratory of Nutrition and Functional Food, Jilin University, Changchun, Jilin, China. Q1
E-mail address: ljb168@sohu.com (J. Liu).

p1226-8453 e2093-4947/$ – see front matter Copyright © 2016, The Korean Society of Ginseng, Published by Elsevier. All rights reserved.
http://dx.doi.org/10.1016/j.jgr.2016.02.007

found that ginsenoside Rb1 can significantly and selectively reduce the hydroxyl radical, which is one of the strongest ROS, with unique molecular mechanisms in a cell-free system. Ni et al [5] have reported that ginsenoside Rb1 exhibits potent neuroprotective effects against oxidative injury induced by tert-butyl hydroperoxide. Jiang et al [6] investigated the effects of ginsenoside Ro in PC12 cells under an anoxic or oxidative environment. Cells treated with ginsenoside Ro had a lower content of ROS, and their survival ratio was higher with a lower apoptosis rate. Ma et al [7] investigated the potential beneficial effect of ginsenoside Rg1 on Schwann cells exposed to oxidative injury, which inhibited the detrimental effect of hydrogen peroxide on cell number and cell viability.

A variety of studies concentrate on yields of extractions and antioxidant mechanisms of individual ginsenoside; however, little is known about the underlying effect of different extraction methods on the biological activity of ginsenosides under the premise of higher yields. Pulsed electric field (PEF) has been used for the extraction of ginsenosides, which showed a higher yield than other common methods [8,9]. Several studies have demonstrated that specific treatments, such as heat processing, can improve the medicinal efficacies of ginsenosides such as antioxidant and anticancer activities, and indicated that the improvement of biological activities was related to the structural change of ginsenosides by heat processing [10–12].

Therefore, our study aimed to compare the antioxidant properties of ginsenosides extracted by PEF and solvent cold soak extraction (SCSE) against H_2O_2-induced oxidative stress. In this study, HEK-293 cells were selected for *in vitro* research. HEK-293 cells are immortalized human embryonic kidney cells, and their metabolic conditions are closer to normal human cells compared with tumor cells, thus showing a more realistic oxidative stress status. The HEK-293 cell line has been widely used for studying *in vitro* oxidative damage [13,14]. Previous researches have reported that H_2O_2 was used as a stable source of free radicals to induce oxidative stress in HEK-293 cells [13,15]. The following experiments were explored to investigate the effects of ginsenosides on oxidative damage, which was measured by determining the cell viability and production of ROS, detected by the MTS assay and laser scanning confocal microscopy, respectively. The studies reported here were performed to expand on previous studies to determine the influence of PEF extraction on the biological fate of ginsenosides following incubation with cells, and their effect on cell viability, intracellular ROS, surface morphology, and cellular antioxidant activity against oxidative damage.

2. Materials and methods

2.1. Materials and chemicals

The dried *P. ginseng* roots were obtained from Ji'an, Jilin province in China. The reference standard ginsenosides (Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd) were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), purity ≥ 98%. Chromatographic grade methanol, acetonitrile, and acetic acid (Fisher Scientific, Waltham, MA, USA) were used as received, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (trolox), fluorescein disodium, 2, 2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2, 2-diphenyl-picrylhydrazyl (DPPH), fluorescence probes 2′, 7′-dichloro dihydro fluorescein diacetate (DCFH-DA), and dimethyl sulfoxide were obtained from Sigma (St. Louis, MO, USA). HEK-293 cell lines were purchased from the American Type Culture Collection. Dulbeco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution (PSS), and MEM nonessential amino acids was obtained from Gibco (CA, USA). The Cell Titer 96 AQueous One Solution Cell Proliferation Assay (MTS) was purchased from Promega Biotechnology Co. Ltd. (Madison, WI, USA). D101 macroporous resin was purchased from Tianjin Pesticide Co. Ltd (Tianjin, China). All other reagents with analytical grade were obtained from Beijing Reagent Company (Beijing, China).

2.2. Preparation of ginsenosides extracted by PEF and SCSE

The dried *P. ginseng* roots were powdered in a pulverizer, and passed through a 120-mesh sieve. The powder were weighed and mixed with 70% (v/v) ethanol–water solution. Subsequently, the mixture were pumped into the PEF system with the conditions of 60 KV/cm electric field intensity, pulse duration of 8 μs, and solid-to-liquid ratio was 1:100 at a flow velocity of 12 mL/min. However, in the SCSE method, the mixture of ginseng powders and ethanol–water solution were added into an erlenmeyer flask and were stirred for 12 h using a magnetic stirrer. When the extractions of the two methods were completed, the ethanol extracts were filtered and evaporated to dryness. The crude saponin fractions were suspended in water and mixed with ether to remove the lipids. Lastly, the ginsenosides were obtained after absorption and disadsorption of D101 macroporous resin and vacuum-rotary evaporation.

2.3. Determination of total ginsenosides contents

The contents of total ginsenosides extracted by PEF and SCSE were determination using the colorimetric method. The standard ginsenoside Re was used to construct a standard curve. The samples were diluted in methanol, and then were mixed with ethanol solution containing 16% vanillin and 77% sulfuric acid solution at 60°C for 15 min. The absorbance of total ginsenosides was detected with spectrophotometry at 544 nm, and the concentration was determined by a standard curve $y = 1.512x + 0.215$ (x, mg/mL, is the content of ginsenoside Re of solution for colorimetric analysis, and y is the absorbance at 544 nm). The experiment was carried out in triplicate and the results were averaged.

2.4. HPLC analysis of ginsenosides extracted by PEF and SCSE

The analysis of ginsenosides extracted by PEF and SCSE were measured according to a protocol described previously with modification [16]. The separation of the ginsenosides was performed on a C-18 analytical column (VP-ODS, 250 mm × 4.6 mm, internal diameter, 5 μm). The detection wavelength was set at 203 nm and the temperature of the column was controlled at 35°C. The gradient elution solvent consisted of acetonitrile (A) and water (B). The process of elution was carried out as follows: 0–24 min, 18–22% A, 82–78% B; 24–26 min, 22–26% A, 78–74% B; 26–30 min, 26–32% A, 74–68% B; 30–50 min, 32–35% A, 68–65% B; 50–55 min, 35–38% A, 65–62% B; and 65 min, 38% A, 62% B. The flow rate was kept at 1.0 mL/min, and the injection volume was 20 μL. Standard ginsenosides (Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd) were mixed and diluted with chromatographic methanol as well as the samples. All solutions were filtered with a nylon filter membrane (0.45 μm) prior to the HPLC analysis.

2.5. DPPH radical scavenging assay

DPPH radical scavenging activity of ginsenosides was measured with a modified version of colorimetric method [17]. One hundred and fifty microliters of 2mM DPPH solution in ethanol was mixed with different concentrations of the sample solution (2 mg/mL, 1 mg/mL, and 0.5 mg/mL) in 96-microwell plates. After the mixture was incubated for 30 min in darkness at room temperature, the

Please cite this article in press as: Liu D, et al., The beneficial effect of ginsenosides extracted by pulsed electric field against hydrogen peroxide-induced oxidative stress in HEK-293 cells, Journal of Ginseng Research (2016), http://dx.doi.org/10.1016/j.jgr.2016.02.007