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Short communication

Protective effect of total phenylethanoid glycosides from *Monochasma savatieri* Franch on myocardial ischemia injury

Mengfan Shi [a], Wenjun He [a], Yanli Liu [a], Xiaoran Li [a], Shilin Yang [a,b], Qiongming Xu [a,*]

[a] College of Pharmaceutical Science, Soochow University, Suzhou 215123, China
[b] Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China

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ABSTRACT

The present study was designed to investigate the cardioprotective effect of total phenylethanoid glycosides from *Monochasma savatieri* Franch (TPG). The data showed that there were mainly four phenylethanoid glycosides isolated and identified from TPG. TPG significantly increased cells viability and inhibited morphological changes on H9c2 cardiomyocytes induced by H_2O_2 or $Na_2S_2O_4$. In addition, TPG significantly decreased T-wave elevation and histopathological changes of heart tissues in myocardial infracted rats induced by isoproterenol. It also significantly reduced the infarct size induced by ligating the coronary artery in rats, increased the activities of antioxidative enzymes superoxide dismutase (SOD), the content of glutathione (GSH), and decreased the leakage of lactic dehydrogenase (LDH), the activities of creatine kinase (CK) and the content of maleic dialdehyde (MDA). In conclusion, these results suggested that TPG from *Monochasma savatieri* Franch might be developed as new natural medicine or food additives with effects of prevention of coronary artery disease due to its significant antioxidant activity.

Introduction

There is growing evidence and interest in natural antioxidants present in vegetables, fruits, beverages and herbs due to their benefits for human health. It is reported that intake of food rich in antioxidants is associated with a lower risk of cardiovascular disease (Gey 1995). Phenylethanoid glycosides are a kind of polyphenolic antioxidants, widely distributed in the plant kingdom, and have been extensively studied for their various biological activities such as hepatoprotective, anti-inflammatory, antinociceptive, sedative effect, antimicrobial and antioxidant activities (Fu et al. 2008). Consistent with this notion, administration of a variety of exogenous antioxidative compounds has been demonstrated to provide protection against oxidative cardiac injury in animal experiments and clinical trials (Reiter and Tan 2003).

Monochasma savatieri Franch, which belongs to Scrophulariaceae, is widely distributed in Southern China (Jiangsu Medical College 1975). It is used in the treatment of common cold, cough, bloody flux, menoxenia, toothache, mammary abscess in traditional Chinese medicine (Fu et al. 2010). Previous studies found that phenylethanoid glycosides were rich source in the whole plant of *M. savatieri*, and 11 phenylethanoid glycosides were isolated (Li et al. 2012). In order to find out if the total phenylethanoid glycosides (TPG) extracted from *M. savatieri* has the potential to be developed as herbal drug with effects of prevention of coronary artery disease, in this paper, the effect of TPG on H9c2 cardiomyocytes viability was evaluated by *in vitro* experiments, as well as the protective effect of TPG on myocardial ischemia by *in vivo* experiments.

Materials and methods

Preparation and quantification of TPG

Extraction and isolation of TPG from *M. savatieri* were shown as our previous research (Li et al. 2012). In brief, the dried plant material (5 kg) was extracted with hot water. The H_2O extract (356.0 g) was passed through a porous polymer gel D101 column, then MeOH–H_2O (3:7) eluate (183.5 g) was chromatographed on a silica gel column (9 cm × 60 cm, 200–300 mesh; Qingdao Marine Chemical Co. Ltd.), eluted with $CHCl_3$–CH_3OH (8:2, 7:3, 6:4, 0:1; each 2 l) and 105.6 g TPG was obtained from the $CHCl_3$–CH_3OH (7:3) part.

The quantification of TPG was performed using an Agilent 1260 HPLC system and a Kromasil C_{18} column (4.6 mm × 250 mm; Akzonobel) was equipped with a Kromasil C_{18} guard column (Akzonobel). TPG was dissolved in methanol to a concentration of 4.0 mg/ml. The mobile phase consisted of (A) methanol and (B) 0.1% formic acid in water at a flow rate of 1.0 ml/min. The concentrations of solvent A in the linear gradient elution was as follows: 35%

* Corresponding author. Tel.: +86 512 69561421; fax: +86 512 65882089.
E-mail address: xuqiongming@suda.edu.cn (Q. Xu).

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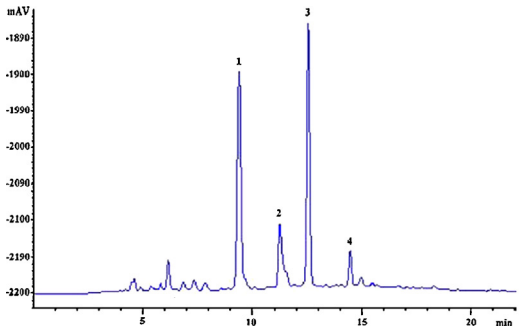

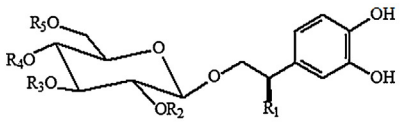

	Name	R1	R2	R3	R4	R5
1	acteoside	H	H	rhamnose	caffeoyl	H
2	savatiside E	OH	rhamnose	H	H	feruloy
3	isoacteoside	H	H	rhamnose	H	caffeoyl
4	savatiside A	OH	rhamnose	H	H	caffeoyl

Fig. 1. HPLC profile of four main constituents of TPG from *M. savatieri*.

at 0–5 min, 35–55% at 6–20 min, and 90% at 21–30 min. Column temperature was controlled at 35 °C.

An Agilent diode array detector (DAD) was set at 330 nm to detect four constituents from TPG: acteoside, savatiside E, isoacteoside, and savatiside A. The reference substances (>95.0% purities) were isolated from *M. savatieri* by ourselves (Li et al. 2012). Four main constituents of TPG from *M. savatieri* were quantified using HPLC-DAD (Fig. 1). The amounts of acteoside, savatiside E, isoacteoside, and savatiside A in the TPG were 224.41, 104.26, 478.18 and 33.87 mg/g, respectively.

Cell viability assay

5×10^4 H9c2 cells/well (the Cell Bank of the Chinese Academy of Sciences Shanghai, China) were seeded into 96-well plates. To evaluate the protective effects of TPG, H9c2 cells were cultured

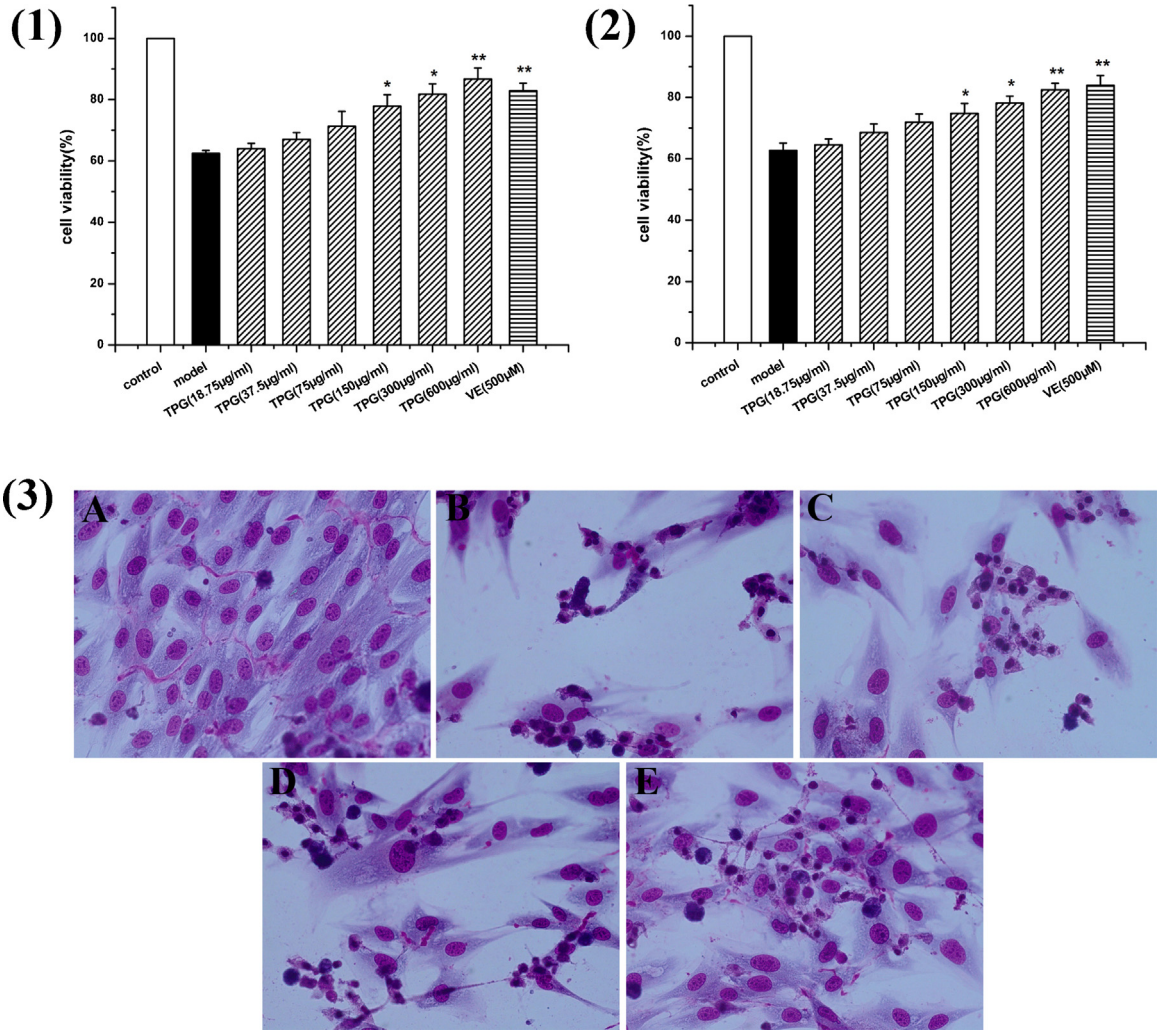

Fig. 2. Protection effects of TPG on H_2O_2 (or $Na_2S_2O_4$)-induced H9c2 cell injury. (1) Cell viability cultured with H_2O_2 (200 μM) for 12 h; (2) cell viability cultured with $Na_2S_2O_4$ (4 mM) for 6 h. Cell viability was determined by MTT assay. Data are expressed as mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$ vs. model group; (3) morphology observations of H9c2 cells. (A) Control: there are almost no abnormal cells observed in the control group; (B) model: a variety number of cells revealing morphological changes including detachment, irregular shape, and nuclear fragmentation; (C–E) TPG (25, 100, and 400 μg/ml): the proportion of injured cells decreasing with increasing TPG concentration; magnification: 400×.