Journal of Ethnopharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Comparison on hypoglycemic and antioxidant activities of the fresh and dried *Portulaca oleracea* L. in insulin-resistant HepG2 cells and streptozotocin-induced C57BL/6J diabetic mice

Jun-fei Gu [a,b,1], Zhi-yin Zheng [a,c,1], Jia-rui Yuan [a,b], Bing-jie Zhao [a,b], Chun-fei Wang [a,d], Li Zhang [a,c], Qing-yu Xu [e], Guo-wen Yin [e], Liang Feng [a,*], Xiao-bin Jia [a,b,c,**]

[a] Key Laboratory of Delivery Systems of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing 210028, Jiangsu, China
[b] College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China
[c] College of Pharmacy, Jiangsu University, Zhenjiang 212013, Jiangsu, China
[d] College of Pharmacy, Anhui University of Chinese Medicine, Hefei 230038, China
[e] Department of Intervention, Cancer Hospital of Jiangsu Province, Nanjing 210009, Jiangsu, China

ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form
16 November 2014
Accepted 1 December 2014

Keywords:
Portulaca oleracea L
Fresh and dried herbs
Hypoglycemic
Antioxidant
Components difference
Diabetes mellitus

ABSTRACT

Ethnopharmacological relevance: Fresh *Portulaca oleracea* L. (family: Portulacaceae; POL) has been used as a folk medicine for the treatment of diabetes mellitus for a long time. More bioactive components with higher activity could be retained in fresh medicinal herbs compared to the dried ones. The present study was conducted to compare different antidiabetic activity between fresh and dried POL, including hypoglycemic and antioxidant activities both in vivo and in vitro. Furthermore, in order to explore which components were responsible for the antidiabetic activity, the difference on chemical components between fresh and dried POL was analyzed and compared.

Materials and methods: Insulin-resistant HepG2 cells induced by insulin were used to evaluate the promoting effect of the fresh and dried POL on glucose utilization in vitro. Streptozotocin (STZ)-induced C57BL/6J diabetic mice were used to compare the differences on hypoglycemic and antioxidant activities of fresh and dried POL, including the fasting blood glucose, glucose tolerance, serum insulin level, malondialdehyde (MDA) level and superoxide dismutase (SOD) activity in vivo. UPLC/Q-TOF–MS method was performed to analyze the difference of antidiabetic components between fresh and dried POL.

Results: Compared with the dried POL extract, the fresh POL extract significantly increased the consumption of extracellular glucose in insulin-resistant HepG2 cells ($P < 0.05$). In STZ-induced C57BL/6J diabetic mice, both fresh and dried extracts decreased markedly the fasting blood glucose (FBG) levels, and improved significantly oral glucose tolerance test (OGTT), as well as enhanced significantly insulin secretion and antioxidative activities ($P < 0.05$; $P < 0.01$). Furthermore, the fresh extract showed stronger antidiabetic activity ($P < 0.05$). The UPLC/Q-TOF–MS analysis results also revealed that the relative contents of polyphenols and alkaloids in the fresh herbs were more abundant than those in the dried POL.

Conclusion: Our results indicated that both fresh and dried POL possessed antidiabetic activities, besides stronger activity was observed in the fresh herb. These findings provided evidence for the application and development of fresh POL in the treatment of diabetes mellitus.

Abbreviations: POL, *Portulaca oleracea* L; STZ, streptozotocin; MDA, malondialdehyde; SOD, superoxide dismutase; FBG, fasting blood glucose; OGTT, oral glucose tolerance test; NA, noradrenaline; DA, dopamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FBS, fetal bovine serum; GC, glucose consumption; ELISA, enzyme linked immunosorbent assay; UPLC/Q-TOF–MS, ultraperformance liquid chromatography/quadrupole-time-of-flight mass spectrometry

* Correspondence to: 100# Shizi Street, Hongshan Road, Nanjing 210028, China. Tel.: +86 25 85637809.
** Corresponding author at: Key Laboratory of Delivery Systems of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing 210028, Jiangsu, China. Tel./fax: +86 25 85637809.
E-mail addresses: wenmoxiushi@163.com (L. Feng), xiaobinjia_nj@126.com (X.-b. Jia).
[1] They contributed equally to this work.

http://dx.doi.org/10.1016/j.jep.2014.12.002
0378-8741/

1. Introduction

Fresh herbs, a special application form of traditional Chinese medicinal herbs, are commonly used as a folk remedy to treat a variety of diseases in China (Jia et al., 2011). Accumulating evidence suggest that fresh herbs have stronger efficacy than dried form and may be optimal for the prevention and treatment of some diseases (Henning et al., 2011; Liang et al., 1999). It has been well shown that much higher content of components with bioactivity are retained in the fresh herbs than the dried ones (Tang et al., 2012; Sanchez-Medina et al., 2007). Some unstable components, such as volatile, flavor (Díaz-Maroto et al., 2003) and total phenolic (Henning et al., 2011), may be destroyed by drying (Díaz-Maroto et al., 2003). Therefore, in order to improve the beneficial use of fresh herbs, the activity and components difference need to be compared in the treatment of some diseases.

Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Rucha et al., 2010; Aslan et al., 2010). Among these plants, *Portulaca oleracea* L. (POL), known as "vegetable for long life" in Chinese folklore, is widely used not only as an edible plant, but also as a traditional Chinese herbal medicine for hypoglycemic. Studies have shown that POL has a variety of bioactivities, such as antibacterial (Zhang et al., 2002), analgesic, anti-inflammatory (Chan et al., 2000), skeletal muscle relaxant and wound-healing (Rashed et al., 2003). POL may be beneficial for type-2 diabetes mellitus patients as an adjunctive and alternative therapy (El-Sayed, 2011a, 2011b). Fresh POL contains abundant catecholamines, phenolic acids and flavonoids, such as noradrenaline (NA), dopamine (DA), caffeic acid, ferulic acid and luteolin, which were considered as the major bioactive components (Ziegler et al., 2012; Strack et al., 2003; Xiang et al., 2005). Research showed that its catecholamines were the effective components for regulating the immune system and preventing diabetes (Del et al., 2011). Moreover, phenolic acids and flavonoids have also been reported to effectively ameliorate diabetes and its complications due to their powerful antioxidant activity (Okezie et al., 2007). However, the antidiabetic acitivities including hypoglycemic and antioxidant activities and the component difference between fresh and dried POL remain unclear.

The aim of this study was to compare the difference between fresh and dried POL on the antidiabetic activity and relevant components variance, and provide evidence for further application of fresh POL on diabetes mellitus treatment.

2. Materials and methods

2.1. Reagents

Roswell Park Memorial Institute-1640 (RPMI-1640) was obtained from Nanjing Keygen biotech CO., LTD (Jiangsu, China); fetal bovine serum (FBS), trypsinase penicillin and streptomycin were obtained from GIBCO Life Technologies (GIBCO, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Amresco (SoIon, OH, USA); blood glucose, MDA, SOD, glycogen kits and the insulin ELISA kit were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China); streptozotoin (STZ) was offered by Sigma Chemical Co. (St. Louis, MO, USA); metformin was obtained from Shanghai Medicinal Company (Shanghai, China); Other chemicals were all reagent grade.

2.2. Plant materials

Fresh POL was collected from Pukou, Jiangsu Province, China in July, 2011. Its botanical identity was confirmed by professor De-kang Wu from Nanjing University of Chinese Medicine. The voucher specimen (no. JTCM-20110811-001) was deposited at Key Laboratory of New Drug Delivery Systems of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine.

2.3. Preparation of plant extracts

Harvested fresh POL (5 kg) was washed by 10 L distilled water to remove impurities. The washed herbs were air-dried and juiced. The herb juice was filtrated. The residues were macerated with 1000 mL distilled water for 24 h until exhaustion, followed by filtration. The filtrate was combined and concentrated in a rotary evaporator at 40 °C under reduced pressure. The concentrated filtrate was subjected to freeze-drying. The yellowish green powder was obtained (35.03 g) and called as "Fresh extract" in this study. The extract yield (w/w) of fresh POL was 0.70%.

Harvested fresh POL (5 kg) was dried under open-air and powdered. The powder was macerated first with 1000 mL distilled water for 24 h at room temperature and then refluxed for three times (1.5 h/time). The extraction was filtrated to remove the impurities. The filtered extracts were concentrated in a rotary evaporator 40 °C under reduced pressure and then subjected to freeze-drying. The resulted yellowish green powder (33.75 g, yield 0.68%, w/w) was referred to as "Dried extract" in this study. Finally, these extracts were reconstituted in double distilled water and filtered with 0.22 μm micropore film, then stored at 4 °C.

2.4. Cell culture

The HepG2 cell line was obtained from the Cell Bank of the Institute of Cell Biology (Shanghai, China). Then cells were cultured in RPMI-1640 containing 10% FBS with penicillin (100 U/mL)/streptomycin (100 μg/mL) in a humidified incubator (5% CO_2) at 37 °C. The medium was renewed every day.

2.5. Glucose consumption

HepG2 cells were seeded into 96-well plates in RPMI-1640 supplemented with 10% FBS and penicillin (100 U/mL)/streptomycin (100 μg/mL), cultured in a humidified incubator (5% CO_2) at 37 °C for 24 h. The insulin-resistant cell model was induced according to the previous reference method (Miyagawa et al., 2010). In brief, HepG2 cells were incubated with a fresh medium containing 1% FBS and 1×10^{-6} mol/L bovine insulin for 24 h. Cells were treated with 10^{-9} mol/L insulin and fresh or dried POL extract (0.25, 0.5, and 1.0 mg/mL, respectively) or metformin (0.086 mg/mL) in RPMI-1640 medium containing 1% FBS. Medium was used as a blank control. After incubation for 24 h, the glucose concentrations in cell supernatant were determined at 505 nm wavelength by the glucose oxidase method. The glucose consumption (GC) was calculated by measuring the glucose concentrations of blank wells and subtracting the remaining glucose in cell plated wells. GC (mmol/L)=[extracellular glucose concentrations of blank wells (mmol/L) −extracellular remaining glucose in cell plated wells (mmol/L)] (Lu et al., 2011).

2.6. MTT assay

To access the cytotoxicity of POL extracts on insulin-responsive cell viability, MTT assay was used to detect the cell viability after the treatment of POL extracts at different concentrations. The prepared cell suspension was seeded into 96-well plates and incubated at 37 °C for 24 h. Then the cells were starved for 12 h. These cells were treated with POL extracts at different concentrations for 24 h. Then the supernatant was discarded and 20 μL MTT (5 mg/mL) was added into each well. Then the cells were

Please cite this article as: Gu, J.-f., et al., Comparison on hypoglycemic and antioxidant activities of the fresh and dried *Portulaca oleracea* L. in insulin-resistant HepG2 cells and.... Journal of Ethnopharmacology (2014), http://dx.doi.org/10.1016/j.jep.2014.12.002