Enhancement of the antioxidant and hypolipidemic activities of *Puerariae radix* by fermentation with *Aspergillus niger*

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Received: 30 April 2018 / Revised: 13 November 2018 / Accepted: 11 December 2018 / Published online: 4 January 2019
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Abstract *Puerariae radix* (PR) is a traditional Chinese food and medicine. In this study, the chemical profile and bioactivities of PR fermented with *Aspergillus niger* (PFA) were investigated. Based on HPLC analysis, PFA chemical profile changed and total phenols increased by 12.5% relative to PR. Consequently, the in vitro antioxidant activity significantly improved. According to the blood lipid analysis in mice, PFA showed a better ability to inhibit the increased blood lipid levels induced by Triton WR-1339 than PR. In a quantitative real-time PCR (qRT-PCR) study, PFA up-regulated cholesterol 7- α hydroxylase (CYP7A1) and low-density lipoprotein receptor mRNA expression, which were 50% and 44.8% higher than the levels induced by high-dose PR. Conversely, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoAR) mRNA expression was down-regulated to 46.8% lower than the levels induced by high-dose PR. The qRT-PCR results suggested that PFA displayed better hypolipidemic activity than PR, due to its superior ability to regulate mRNA expression.

Keywords Antioxidant · *Aspergillus niger* · Hypolipidemic · *Puerariae radix* · Solid fermentation

Introduction

Currently, due to the growing demand for healthy and functional foods, edible and medicinal plants have attracted increasing attention. *Pueraria lobata* (Willd.) Ohwi (Leguminosae) is a plant with a long history in Asia. The roots of *P. lobata* are used as a superior animal feed, a natural health food and effective medicines in Asian countries (Xia et al., 2013). Many studies have revealed that *Puerariae radix* (PR) is rich in isoflavones and starch and can prevent and treat cardiovascular disease, decrease blood sugar and ameliorate menopausal syndrome (Wook et al., 2013; Xie et al., 2012). Hence, functional food development of PR is very promising.

Microbial fermentation has been used to make foods and drugs for thousands of years in China; this technique can enhance known therapeutic effects, decrease toxicity and produce additional functions (Xia and Yang, 2010). In modern industrial applications, this natural process can be used to produce desired products, such as pigments, antioxidants, and other molecules, in a controlled manner. These products are more popular among consumers than those produced by chemical synthesis (Couto and Sanromán, 2006). In particular, researchers have attempted to obtain new functions from traditional medicines or foods by microbial fermentation in recent years (Chen et al., 2013; Ni et al., 2015).

Aspergillus niger (*A. niger*) is an edible fungus used and distributed worldwide. It is generally regarded as safe by the United States Food and Drug Administration and is used for biotransformation, waste treatment and citric acid production (Bansal et al., 2012; Papagianni, 2007; Schuster et al., 2002). *A. niger* has been used for the biotransformation of many kinds of compounds, such as isoflavones, saponins, and steroids (Miyazawa et al., 2006; Parshikov

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et al., 2015). Hence, *A. niger* should be an appropriate strain to conduct solid fermentation in this study. During fermentation, *A. niger* produces various enzymes that are needed in plant fiber degradation, and this promotes the release of active compounds in PR. At the same time, starch and fiber in PR supply carbon source for *A. niger* growth.

The aim of this study was to add value to PR through solid fermentation. Recently, PR has been added to many types of functional foods, such as tea, wine and vinegar, via fermentation to improve their health characteristics (Zhang, 2013). To the best of our knowledge, there are limited reports about PR fermentation by *A. niger*. In addition, most existing studies have focused on flavors and production techniques, while bioactivities are rarely investigated (Chen and Zhao, 2015). PR possesses high antioxidant activity (Guerra et al., 2000; Yu et al., 2004), and is used with other medicines to treat hyperlipidemia (Liu et al., 2011; Xie et al., 2012). We focused on changes in the bioactivity of PR before and after solid fermentation with *A. niger*. Hence, we compared the antioxidant activity and hypolipidemic effect between unfermented PR and fermented PR in this work. This study will provide foundational data for further applications.

Materials and methods

Chemicals and reagents

Glutathione (GSH) assay kit (No. 20150604), malondialdehyde (MDA) assay kit (No. 20150604), superoxide dismutase (SOD) kit (No. 20150604), catalase (CAT) assay kit (No. 20150604), total cholesterol (TC) assay kit (No. 20150522), triglyceride (TG) assay kit (No. 20150522), low-density lipoprotein (LDL) assay kit (No. 20150516), and high-density lipoprotein (HDL) assay kit (No. 20150516) were obtained from Nanjing Jiancheng Bio-engineering Co. (Nanjing, Jiangsu, China). All chemicals and solvents were the highest commercially available grade.

Microorganism and materials

PR was purchased in the medicine market (Chengdu, Sichuan, China) and identified by Professor Zhang Hao (Sichuan University). *Aspergillus niger* CICC 40338 was provided by China Center of Industrial Culture Collection (CICC) and stored at -20°C before cultivating.

Preparation of cultures and solid-state fermentation

Freeze-dried spores of *A. niger* were recovered on potato dextrose agar medium for 5 days at 30°C and then cultured in yeast extract peptone dextrose fluid medium at 160 rpm/min and 30°C for 3 days.

Then, 25 g PR was mixed with 25 mL distilled water in a 250 mL flask. The substrate was autoclaved at 121°C for 20 min, then stirred evenly and cooled down naturally. The sterilized PR was inoculated with 10% (v/w) prepared seed broth and statically cultured at 30°C for 10 days. After 10 days, the fermentation products were dried at 50°C for 24 h and ground by a mill to fine powder (100 mesh).

Contents of six principal isoflavones

First, samples were accurately weighted out to 1 g and extracted with 15 mL 70% ethanol water solution by ultrasonic extraction for 30 min. The extract was assayed by high performance liquid chromatography (HPLC). The remaining supernatant was diluted to five concentrations and stored at 4°C for further analysis of antioxidant activities. Second, standard solutions of isoflavones at different concentrations were prepared. All solutions were filtered through a polytetrafluoroethylene filter ($0.45\ \mu\text{m}$).

The HPLC analysis was performed on a liquid chromatograph system that consisted of a LC-10Atvp pump, a CTO-10Asvp column oven, a SCL-10Avp system controller, a SPD-10Avp detector and a Class-vp chromatographic work station (LC-10Atvp; Shimadzu, Tokyo, Japan). The chromatography column Kromasil C18 ($4.6\ \text{mm} \times 250\ \text{mm}$, $5\ \mu\text{m}$, Akzo Nobel, Bohus, Sweden) was used for sample separation. The solvent flow condition was described in our previous study (Huang et al., 2017). Briefly, the mobile phase was composed of water (solvent A) and methanol (solvent B), with gradient elution as follows: 0–10 min, 25% B; 10–20 min, 25–30% B; 20–60 min, 30–100% B; 60–70 min, 100–25% B. The flow rate was 0.5 mL/min. The temperature of the column oven was set at 35°C . The wavelength detector was set at 250 nm, and the injection volume of each sample was 5 μL . Contents are expressed as mg/g dry weight (mg/g DW).

Total phenolic contents (TPC) and antioxidant activities in vitro

The experimental procedures were described in our previous study (Huang et al., 2017). TPC is expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW). The total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl ($\cdot\text{OH}$) scavenging activities are both expressed as mg ascorbic acid equivalents/g of

sample (mg AAE/g of sample). Moreover, ferric reducing antioxidant power (FRAP) is expressed as mmol Fe²⁺/g of sample. Vitamin C was the positive control.

Hypolipidemic effect in mice

Eighty male Kunming mice (25–30 g) were obtained from Dashuo Experiment Animal Co. (Chengdu, Sichuan, China). Before the experiments, the mice were acclimatized under laboratory conditions for 1 week. The mice were housed in a controlled temperature (22 ± 1 °C) and relative humidity ($40 \pm 10\%$) with a reverse 12 h light/dark cycle. They were provided with standard food and water ad libitum. All the experiments with animals were carried out in accordance with the guidelines of the institutional animal ethical committee (Number: SCU2016-1204). The samples were prepared as follows: PFA and PR were weighted out at 1 g and extracted with 15 mL 70% ethanol water solution by ultrasonic extraction for 30 min. The extracts were evaporated at 45 °C, lyophilized and then dissolved with 10 mL normal saline. Finally, the extracts were diluted with normal saline to 50 mg/mL (crude drug, low dose) and 100 mg/mL (crude drug, high dose).

The mice were randomly divided into nine groups, and each group included 10 mice. According to the method described by Chen and Li (2007), the mice were fasted for 12 h prior to initiation of the experiment. Triton WR-1339 (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in normal saline to 30 mg/mL. All mice were injected with Triton WR-1339 or normal saline. Twelve hours after the injection, all mice were treated as shown in Table 1, and they sacrificed at 24 h after Triton WR-1339 injection. The serum and liver samples were collected and stored at -70 °C for further analysis.

The serum lipid levels (TC, TG, HDL and LDL), GSH and MDA levels and SOD and CAT activities in the liver were detected by using commercial reagent kits purchased from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, Jiangsu, China) according to the instruction manuals.

RT-PCR

The mRNA expression of LDLR, CYP7A1, and HMGCoAR was determined from liver tissue. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The RNA was reverse transcribed into cDNA using the PrimeScript RT Reagent Kit (RR047A; Takara, Dalian, Liaoning, China). The mRNA expression levels of LDLR, CYP7A1, and HMGCoAR were evaluated by qRT-PCR analysis using the SYBR Premix Ex Taq II Kit (RR820A; Takara, Dalian, Liaoning, China). qRT-PCR was performed on ThermoFisher PIKORed 96 system (ThermoFisher, Waltham, MA, USA). The primer sequences are listed in Table 2. Target mRNA expression in each sample was normalized to the housekeeping gene β -actin. The $2^{-\Delta\Delta CT}$ method was used to calculate relative mRNA expression levels.

Statistical analysis

The data obtained were analyzed by using Statistical Product and Service Solutions (SPSS) V.19 (SPSS Inc., Chicago, IL, USA) and are presented as the mean $\pm$ SD. Statistically significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by LSD tests and Dunnett's tests. $p < 0.05$ was considered statistically significant.

Table 1 The groups information and administrations

Group	Treatment	
	Intraperitoneal injection	Oral administration
Normal control	Normal saline	Normal saline
Model control	300 mg/kg Triton WR-1339	Normal saline
PFA-low	300 mg/kg Triton WR-1339	PFA 0.5 g/kg, b. w.
PFA-high	300 mg/kg Triton WR-1339	PFA 1 g/kg, b. w.
PR-low	300 mg/kg Triton WR-1339	PR 0.5 g/kg, b. w.
PR-high	300 mg/kg Triton WR-1339	PR 1 g/kg, b. w.
Lovastatin	300 mg/kg Triton WR-1339	lovastatin 10 mg/kg, b. w.
PFA-NS	Normal saline	PFA 1 g/kg, b. w.
PR-NS	Normal saline	PR 1 g/kg, b. w.

The dosage was calculated with crude materials

PFA Puerariae radix fermented by *Aspergillus niger*; PR Puerariae radix; NS Normal saline; b. w. body weight

Table 2 The sequences of the primers used for real-time PCR

Gene	Forward	Reverse
β -actin	5'-GAAGATCAAGATCATTGCTCCT	5'-TACTCCTGCTTGCTGATCCA
CYP7A1	5'-GCCCTGAAGCAATGAAAGCAGCCTCT	5'-GAGAGCCGCAGAGCCTCCTTGATGA
HMGC α AR	5'-TGACGATGGCAGGACGCAACCTCTAT	5'-CACCTTGGCTGGAATGACGGCTTCAC
LDLR	5'-CCTGGAAGGCAGCTACAAGTGTGAGT	5'-CGTGGCGGTTGGTGAAGAGCAGATAG

Results and discussion

Contents of isoflavones determined by HPLC

Isoflavones are important indicators used to evaluate the quality of PR. In this work, several representative isoflavones were chosen to reflect the contents of isoflavones in the samples. We found that the species and contents of the PR compounds were changed after fermentation. These changes may alter the bioactivities of PR. Glycosides, such as daidzin, were hydrolyzed to the corresponding aglycones. However, most of the principal compounds in PR, such as puerarin, were reserved after fermentation. The results agreed with the report of Choi and Ji (2005) showing that *O*-glycoside but not *C*-glycoside isoflavones in PR are effectively hydrolyzed by microorganisms. Puerarin represents *C*-glycoside isoflavones and daidzin represents *O*-glycoside ones. As shown in Table 3, the total contents of the major isoflavones in PFA showed a slight increase compared with those in unfermented PR ($p < 0.05$). The increases in the contents of puerarin and 3'-OH puerarin were probably due to the decrease of mirificin and 3'-OCH₃ puerarin and the consumption of non-isoflavones (including

protein, carbohydrate). However, glycoside compounds such as daidzin strongly decreased from 7.31 ± 0.22 mg/g DW to 0.31 ± 0 mg/g DW, and homologous aglycones such as daidzein increased from 0.66 ± 0.03 mg/g DW to 7.23 ± 0.09 mg/g DW. The results were consistent with those of previous studies (Lee et al., 2015; Raimondi et al., 2009). In addition, aglycones have higher antioxidant activity and better absorption by the human intestinal tract than isoflavone glucosides, owing to their better lipid solubility (Chi and Cho 2016; Marazza et al., 2012). Hence, fermentation by *A. niger* may enhance PR bioactivities. In addition, the molecular weight of compounds such as daidzin (416), which was hydrolyzed to daidzein, was much higher than that of daidzein (254). If all daidzin was transformed to daidzein, the content of daidzein in PFA should be 4.46 mg/g DW. In fact, the total daidzein content was 7.23 mg/g DW in PFA. Therefore, the moles of isoflavones in unit weight increased. This increase will enhance the bioactivities of PR. The extra daidzein content may be due to other glucosides with the same aglycones as daidzin or certain precursors.

Contents of total phenolics

Studies have demonstrated the high correlation between TPC and antioxidant activity (Paixão et al., 2007). Hence, determination of TPC is necessary. In this study, the TPC of samples was detected with the Folin-Ciocalteu method and is expressed as gallic acid equivalents (mg GAE/g DW). The data showed that the phenolic contents increased from 44.39 ± 0.02 mg GAE/g DW (unfermented PR) to 49.95 ± 0.04 mg GAE/g DW (PFA). Some reports have suggested that substances used as Chinese medicines may be concentrated during microorganism growth, due to the consumption of proteins, carbohydrates and other nutrients (Chandrasekara and Shahidi, 2012).

Antioxidant capacity

Several antioxidant capacity assays with different mechanisms were performed to measure the antioxidant capacities of the samples, and the results are displayed in Table 4.

Table 3 The contents of main isoflavones of samples

Isoflavones	Contents (mg/g DW)	
	PFA	PR
3'-OH puerarin	3.58 ± 0.10^a	3.35 ± 0.12^b
Puerarin	39.38 ± 1.03^a	36.32 ± 0.67^b
Mirificin	6.75 ± 0.01^a	8.31 ± 0.51^b
3'-OCH ₃ puerarin	6.85 ± 0.09^a	7.40 ± 0.35^b
Daidzin	0.31 ± 0^a	7.31 ± 0.22^b
Daidzein	7.23 ± 0.09^a	0.66 ± 0.03^b
Total	64.1 ± 0.57^a	63.35 ± 0.18^b

The data is expressed as mean $\pm$ SD, $n = 6$

DW dry weight of sample; PFA Puerariae radix fermented by *Aspergillus niger*; PR Puerariae radix

Values with the different letters were significantly different at the level of 0.05

Table 4 Antioxidant activities of PFA and PR in vitro

Samples	Total antioxidant activity (mg AAE/g of sample)	DPPH (mg AAE/g of sample)	·OH (mg AAE/g of sample)	FRAP (mmol Fe ²⁺ /g of sample)
PFA	24.327 ± 2.310 ^a	12.739 ± 0.652 ^a	256.215 ± 12.134 ^a	0.112 ± 0.063 ^a
PR	19.415 ± 0.521 ^b	10.576 ± 0.517 ^b	133.433 ± 11.369 ^b	0.110 ± 0.037 ^a

The data is expressed as the mean ± SD, n = 6

AAE ascorbic acid equivalents; PFA Puerariae radix fermented by *Aspergillus niger*; PR Puerariae radix

Values with the different letters were significantly different at the level of 0.05

In the total antioxidant activity assay, the activity of PFA was over 25% superior to that of PR. Similarly, PFA extracts exhibited stronger DPPH scavenging activity and hydroxyl radical scavenging activity than PR. These antioxidant activities increased by 20.4% and 92.0% after fermentation, respectively. However, the FRAP of PFA showed no significant difference from that of PR ($p > 0.05$).

Although different analysis methods have different reaction mechanisms, the results above indicated that solid fermentation with *A. niger* enhanced the antioxidant activities of PR. The results have three possible explanations. First, daidzein has higher antioxidant activity than daidzin (Chen et al., 2011). Second, the antioxidant activity of 3'-OH puerarin is 20 times higher than that of puerarin, and thus, increased 3'-OH puerarin can enhance PR antioxidant activity (Ye et al., 2007). Finally, the contents of isoflavones and phenols, which are the major antioxidant ingredients in PR, were increased by solid fermentation. These findings are consistent with the observations by Dulf et al. (2017).

Hypolipidemic activity

Injection of Triton WR-1339 induces acute hyperlipidemia. This procedure is a fast and convenient method to screen potential hypolipidemic drugs. PR is a traditional Chinese medicine, that is used with other herbs to treat hyperlipidemia (Xie et al., 2012). Thus, we explored the hypolipidemic effect of PFA, which may perform better than PR, as shown in the previous in vitro studies.

In this study, blood lipid levels and related enzyme activities were assessed, and the results are displayed in Table 5. After the injection of Triton WR-1339, the TC, TG and LDL levels in serum of the model group were significantly increased ($p < 0.01$), and the HDL level was slightly decreased compared with that of the normal group ($p < 0.05$). These findings indicated that the acute hyperlipidemia model was successfully established. As shown in Table 5, PFA and PR treatments reduced the changes in blood lipid parameters induced by Triton WR-1339.

Specifically, TC, TG and LDL levels in the PFA- and PR-treated groups were significantly reduced, and HDL levels were enhanced relative to those in the model group ($p < 0.05$). Both PFA and PR improved blood lipid parameters in a dose-dependent manner in hyperlipidemic mice. Furthermore, PFA was better at regulating blood lipid levels than PR at a high dose, especially for TC (15.9% lower than PR) and TG (10.8% lower than PR). Both PFA and PR are quite safe at high doses.

Hyperlipidemia results in dysfunction between oxidation and antioxidation. Hence, many oxygen free radicals and high levels of MDA will be produced. These changes induce cardiac-cerebral vascular disease, liver damage and aging (Shao et al., 2016). The SOD and CAT activities indicate the ability to scavenge oxygen free radicals. The GSH and MDA contents reflect the current degree of lipid peroxidation. In this work, injection of Triton WR-1339 strongly attenuated SOD and CAT activities in model group ($p < 0.01$). In addition, the GSH content decreased, and the MDA content increased ($p < 0.01$, $p < 0.01$). In the PFA-treated groups, the decreased SOD and CAT activities and GSH level were partly recovered, and the elevated MDA level was slightly decreased, similar to the PR-treated groups. Hence, treatments with PFA and PR reduced lipid peroxidation and enhanced the activities of antioxidant enzymes at both low and high doses. In addition, PFA at a high dose is more effective at increasing GSH and decreasing MDA levels than PR ($p < 0.01$, $p < 0.05$). Hence, PFA is a better choice than PR to alleviate lipid peroxidation induced by hyperlipidemia.

The antihyperlipidemia results were consistent with the antioxidant results in vitro. PFA exhibited superior antihyperlipidemic activity relative to PR. There may be three explanations for the results. First, oxidative stress plays a major role in endothelial damage and atherosclerosis. Oxidants can oxygenate LDL to oxidized LDL and aggravate the accumulation of cholesterol. Hence, antioxidant activity plays an important role in the hypolipidemic effect. PFA has higher antioxidant activity than PR, as observed in a previous in vitro study. Second, the major bioactive ingredients in PFA, similar to those in PR, are

Table 5 Results of different groups on blood lipid levels and antioxidant parameters of liver

Group	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (μ mol/g protein)	MDA (μ mol/g protein)
Normal control	4.22 $\pm$ 0.30 ^a	2.34 $\pm$ 0.05 ^a	1.41 $\pm$ 0.02 ^a	0.46 $\pm$ 0.01 ^a	127.6 $\pm$ 8.2 ^a	17.2 $\pm$ 0.5 ^a	22.1 $\pm$ 0.2 ^a	1.8 $\pm$ 0.6 ^a
Model control	6.20 $\pm$ 0.45 ^b	3.18 $\pm$ 0.12 ^b	1.22 $\pm$ 0.07 ^b	0.95 $\pm$ 0.06 ^b	83.2 $\pm$ 5.4 ^b	13.6 $\pm$ 0.4 ^b	7.2 $\pm$ 0.8 ^b	3.2 $\pm$ 0.3 ^b
PFA-low	4.51 $\pm$ 0.53 ^a	2.05 $\pm$ 0.11 ^a	1.35 $\pm$ 0.05 ^b	0.76 $\pm$ 0.03 ^c	113.1 $\pm$ 6.5 ^c	15.5 $\pm$ 0.3 ^c	12.5 $\pm$ 0.4 ^c	3.0 $\pm$ 0.2 ^b
PFA-high	3.96 $\pm$ 0.21 ^a	1.74 $\pm$ 0.06 ^c	1.37 $\pm$ 0.04 ^c	0.57 $\pm$ 0.03 ^c	119.3 $\pm$ 7.6 ^a	16.5 $\pm$ 0.2 ^a	17.6 $\pm$ 1.0 ^c	2.4 $\pm$ 0.2 ^a
PR-low	4.80 $\pm$ 0.33 ^a	2.25 $\pm$ 0.22 ^a	1.30 $\pm$ 0.04 ^b	0.73 $\pm$ 0.10 ^c	103.0 $\pm$ 4.2 ^c	15.1 $\pm$ 0.4 ^c	12.7 $\pm$ 0.8 ^c	2.8 $\pm$ 0.1 ^b
PR-high	4.71 $\pm$ 0.22 ^a	1.95 $\pm$ 0.16 ^a	1.34 $\pm$ 0.02 ^c	0.60 $\pm$ 0.02 ^c	114.2 $\pm$ 6.7 ^a	16.4 $\pm$ 0.3 ^a	15.2 $\pm$ 0.2 ^c	2.7 $\pm$ 0.2 ^b
Lovastatin	4.31 $\pm$ 0.32 ^a	1.98 $\pm$ 0.20 ^a	1.27 $\pm$ 0.01 ^b	0.52 $\pm$ 0.01 ^c	107.2 $\pm$ 8.2 ^c	15.4 $\pm$ 0.5 ^c	15.2 $\pm$ 0.5 ^c	2.4 $\pm$ 0.3 ^c
PFA-NS	3.94 $\pm$ 0.17 ^a	1.82 $\pm$ 0.11 ^c	1.45 $\pm$ 0.12 ^a	0.46 $\pm$ 0.03 ^a	128.5 $\pm$ 5.5 ^a	17.5 $\pm$ 0.6 ^a	23.3 $\pm$ 0.6 ^a	2.3 $\pm$ 0.1 ^a
PR-NS	4.07 $\pm$ 0.52 ^a	1.75 $\pm$ 0.21 ^c	1.41 $\pm$ 0.08 ^a	0.43 $\pm$ 0.04 ^a	127.7 $\pm$ 8.3 ^a	18.3 $\pm$ 0.8 ^a	22.4 $\pm$ 0.5 ^a	2.3 $\pm$ 0.2 ^a

The data is expressed as mean $\pm$ SD, n = 6

PFA Puerariae radix fermented by *Aspergillus niger*; PR Puerariae radix; NS Normal saline

Values with the different letters were significantly different at the level of 0.05, compared with normal and model group

isoflavones. In addition, isoflavones in PR have been shown to have hypolipidemic effects (Park et al., 2009; Wook et al., 2013). The contents of isoflavones and phenols per gram of PFA were higher than those in PR. Third, the ratio of aglycones in PFA was higher than that in PR. Aglycones have better lipid solubility than glycosides (Lee et al., 2015; Marazza et al., 2012). Hence, more aglycones than glycosides will be absorbed from the intestinal tract

into the blood circulation. Therefore, PFA has stronger hypolipidemic effects.

RT-PCR

In order to search the possible mechanism of PFA and PR in hyperlipidemic activity, a qRT-PCR study was done in mice liver. The CYP7A1 enzyme converts cholesterol into

Fig. 1 Effects of Puerariae radix fermented by *Aspergillus niger* (PFA) and Puerariae radix (PR) on mRNA expression in the mouse liver. **(A)** Cholesterol 7 α -hydroxylase (CYP7A1), **(B)** 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGCoAR), and **(C)** low density lipoprotein receptor (LDLR). Each value is expressed as the

mean $\pm$ SD (n = 3). ^ap < 0.05 versus model control, ^bp < 0.05 versus normal control. C: normal control; M: model control; PR-L: PR treatment at 0.5 g/kg; PR-H: PR treatment at 1 g/kg; PFA-L: PFA treatment at 0.5 g/kg; PFA-H: PFA treatment at 1 g/kg; Lova: lovastatin treatment at 10 mg/kg

7-hydroxycholesterol, which is an important step in the bile acid biosynthesis pathway in the liver (Russell, 2003). Hence, the higher expression of CYP7A1 mRNA leads to faster consumption of cholesterol. In addition, the plasma cholesterol concentration is regulated mainly by the LDL receptor pathway, in which circulating LDL is taken into cells by receptor-mediated endocytosis (Brown and Goldstein, 1986). Moreover, 3-hydroxy-3-methylglutaric acid (HMG) is a competitive inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCoAR) and strongly reduces cholesterol biosynthesis both in vitro and in vivo. As is known to all, lovastatin is the first approved inhibitor of HMG-CoA reductase in the world, so it was chosen as the positive control in the study (Padova et al., 1982; Tobert, 2003). As shown in Fig. 1, the expression of CYP7A1 mRNA and LDLR mRNA was restrained by Triton WR-1339. Compared with the model group, treatment with PR and PFA up-regulated mRNA expression in a dose-dependent manner, especially in the PFA-H groups ($p < 0.01$). On the other hand, high-dose PFA and lovastatin significantly inhibited the expression of HMGCoAR mRNA ($p < 0.01$, $p < 0.01$). Hence, PFA showed better performance than PR in regulating the expression of lipid metabolism-related mRNA. It is the further evidence that PFA has better hyperlipidemic activity than PR.

In conclusion, microbial solid fermentation is a commercial and effective method to alter the functions of crude plant medicines. In our study, *Aspergillus niger* CICC 40338 could change the ingredients of PR and improve its antioxidant activity in vitro and its hypolipidemic effect in vivo. PFA is a promising functional food that has anti-aging, antioxidant and antihyperlipidemia effects.

Acknowledgements This research was financially supported by Sichuan Science and Technology Support Program (No. 2014S20131).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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