Journal of Ethnopharmacology 139 (2012) 287–293

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Lipid regulation effects of Polygoni Multiflori Radix, its processed products and its major substances on steatosis human liver cell line L02

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ARTICLE INFO

Article history:
Received 5 July 2011
Received in revised form 19 October 2011
Accepted 12 November 2011
Available online 20 November 2011

Keywords:
Polygonum multiflorum
Non-alcoholic fatty liver disease (NAFLD)
Lipid regulation effects
Human liver cell line L02

ABSTRACT

Ethnopharmacological relevance: Raw and processed Polygoni Multiflori Radix (PMR) are used in the prevention and treatment of non-alcoholic fatty liver disease (NAFLD), hyperlipidemia or related diseases. However, few researches compared the activities of raw and processed PMR on lipid metabolism regulation. Moreover, the active substances of *Polygonum multiflorum* are still not clearly elucidated.
Materials and methods: In this research, a sensitive, accurate and rapid *in vitro* model, steatosis hepatic L02 cell, was applied to compare the relative activities of raw and processed PMR on lipid metabolism regulation. Furthermore, the lipid regulation activities of emodin, physcion and 2,3,5,4′-tetrahydroxy-stilbene-2-*O*-β-D-glucoside (TSG) were evaluated. The steatosis L02 cells were obtained after cultured with 1% fat emulsion–10% fetal bovine serum (FBS)–RPMI 1640 medium for 48 h. Contents of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) in L02 cells are evaluated after exposure.
Results: The intracellular TG contents were increased from 16.50 ± 1.29 mmol/L to 34.40 ± 1.36 mmol/L in steatosis L02 cells, while the intracellular contents of TC were increased from 5.07 ± 1.80 mmol/L to 11.79 ± 0.54 mmol/L. Water extract of raw PMR showed much remarkable TG-regulation and TC-regulation effects than its processed products. Emodin displayed the best TG regulation activity while TSG showed the best TC regulation activity. At the same time, the exposure of emodin and physcion could reduce the LDL-C contents in steatosis L02 cells.
Conclusions: On account of these *in vitro* results, raw PMR might have more satisfactory effects in clinic treatment of NAFLD or hyperlipidemia characterized by the elevation of cholesterol than processed PMR.

1. Introduction

After recently being recognized as a feature of the metabolic syndrome, fatty liver has evolved as a critical player in the pathogenesis of hyperlipidemia. Fatty liver is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (abnormal retention of lipids within a cell). The liver plays a major role in the metabolism and excretion of both endogenous and exogenous substances (Ronald et al., 1995). The hepatocytes play important role in the distribution, biosynthesis, transferring and removal of triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and other related lipoproteins. In normal human liver, the mean contents of total cholesterol and triglyceride are 3.9 and 19.5 mg/g wet weight, respectively. Traditionally, liver fat content >50 mg/g (5% by wet weight) is diagnostic of hepatic steatosis (Kwiterovich et al., 1970; Szczepaniak et al., 2005).

By considering the contribution by alcohol, fatty liver may be termed alcoholic steatosis or non-alcoholic fatty liver disease (NAFLD). Development of NAFLD comes from an imbalance between the influx and production of fatty acids and the use of fatty acids for oxidation or secretion. The progress of NAFLD is usually characterized by the morphologic changes of hepatocytes and the hepatic triglyceride contents.

On account of the effectiveness and popular prices, the prevention and treatment of NAFLD and hyperlipidemia by traditional

Abbreviations: DMSO, dimethylsulfoxide; FBS, fetal bovine serum; G_0 medium, 0.2% FBS–RPMI 1640 medium; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; HPLC-DAD, high performance liquid chromatography coupled with diode array detector; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; M1, 1% fat emulsion–10% FBS–RPMI 1640 medium; M2, 50% FBS–RPMI 1640 medium; NAFLD, non-alcoholic fatty liver disease; PMR, Polygoni Multiflori Radix; PMRP, Polygoni Multiflori Radix Praeparata; PMRP-A, Polygoni Multiflori Radix Praeparata (steamed solely); PMRP-B, Polygoni Multiflori Radix Praeparata (steamed with black soybean decoction); RPMI 1640, Roswell Park Memorial Institute medium 1640; TC, total cholesterol; TG, triglyceride; TSG, 2,3,5,4′-tetrahydroxy-stilbene-2-*O*-β-D-glucoside.

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doi:10.1016/j.jep.2011.11.022

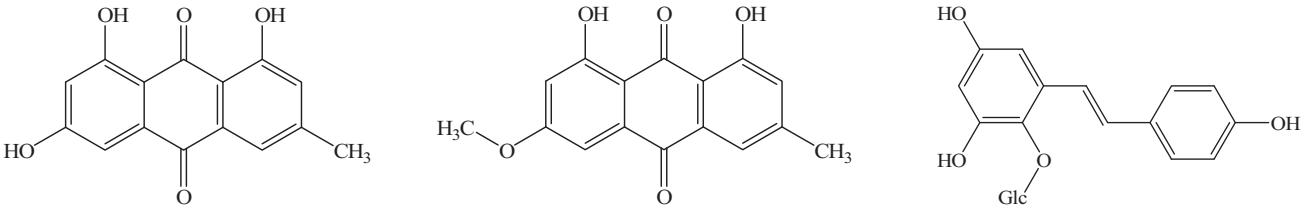

Fig. 1. Structures of emodin, physcion and TSG. TSG: 2,3,5,4′-tetrahydroxy-stilbene-2-*O*-β-D-glucoside.

Chinese medicine attract more and more attention worldwide (Chen et al., 2011; Guo et al., 2011). Polygoni Multiflori Radix (PMR, Heshouwu in Chinese) and Polygoni Multiflori Radix Praeparata (PMRP, Zhiheshouwu in Chinese), originated from the root of *Polygonum multiflorum* Thunb., are applied in the treatment of NAFLD and hyperlipidemia in oriental counties for centuries (Commission of Chinese Pharmacopoeia, 2010). *Polygonum multiflorum* ranked the fifth most frequently used crude drugs in prevention and treatment of hyperlipidemia and NAFLD (Zhang and Chen, 2007). However, PMRP has higher frequency of occurrences than PMR in traditional Chinese medicine (TCM) prescriptions aiming at lipid metabolism regulation. For instance, three prescription preparations containing PMRP are recorded for the treatment of hyperlipidemia in Pharmacopoeia of People's Republic of China (2010 edition) (Commission of Chinese Pharmacopoeia, 2010): Xuezhining Wan, Xuezhiling Pian and Shouwu Wan. Nevertheless, only one preparation, Zhengxin Jiangzhi Pian, containing PMR is recorded. Meanwhile, few researches focused on the relative activities of PMR and PMRP and whether the widespread use of PMRP in the treatment of NAFLD, hyperlipidemia and related diseases is rational or not.In addition, the active substances of *Polygonum multiflorum* are still not clearly elucidated. The activities comparison between TSG and anthraquinones have not been detailed researched, although both stilbene (mainly 2,3,5,4′-tetrahydroxy-stilbene-2-*O*-β-D-glucoside, TSG) (Gao et al., 2007; Luo et al., 2008) and anthraquinones (mainly emodin and physcion) (Dong et al., 2005; Li et al., 2008; Zhao et al., 2009) show some lipid metabolism regulation activity.

In this research, we develop a sensitive, accurate and rapid *in vitro* model to compare the relative activities of raw and processed PMR on lipid metabolism regulation. L02 hepatocytes, the isolated normal human hepatic parenchymal cell, are used in this research. This cell line could maintain characteristics and ultrastructure of normal liver cells after continuous passage (Ye et al., 1980; Li et al., 2007). L02 cell line has been shown to express many specific liver cell functions and could be used in many fields such as activity (Liu et al., 2006; Jin et al., 2009; Liu and Zhang, 2009; Chen et al., 2010; Ye et al., 2011), toxicity (Wang et al., 2008; Yuan et al., 2009) and artificial liver research (Bao-san et al., 2008). The steatosis L02 cells are widely used in the lipid metabolism regulation researches (Gomez-Lechon et al., 2007; Shen et al., 2008; Pan et al., 2010; Zhang et al., 2011), especially for traditional Chinese medicine and their active compounds, such as saponin (Zhang et al., 2011), ursolic acid (Shen et al., 2008) and curcumin (Pan et al., 2010).

2. Materials and methods

2.1. Chemicals

TSG, emodin and physcion were purchased from National Institute for the Control of Pharmaceutical and Biological Products, China. The purities of all the standards were not less than 98%. Structures of them were listed in Fig. 1.

Lovastatin (Jiangsu Dayang Pharmaceutical Co., Ltd, China) and fenofibrate (Laboratoires Fournier S.A., France) were used as positive controls for cholesterol-lowering and triglyceride-lowering, respectively. Fetal bovine serum (FBS) was purchased from Hyclone. Fat emulsion for human use was purchased from Sichuan Guorui Pharmaceutical Co., Ltd, China. The fat emulsion (each 250 mL) contained 50 g of refined soybean oil, 5.5 g of glycerol and 3 g of refined lecithin.

2.2. Processing procedure of Polygoni Multiflori Radix

Raw Polygoni Multiflori Radix was collected in Luquan County of Yunnan Province by the authors in June 2008 and identified as the root of *Polygonum multiflorum* Thunb. by Prof. Ronghua Zhao, Yunnan University of Traditional Chinese Medicine. Voucher specimens were deposited in the Herbarium of Pharmacognosy, Yunnan University of Traditional Chinese Medicine. PMRP-A was steamed from PMR solely. PMRP-B was steamed with black soybean decoction according to the procedure recorded in Pharmacopoeia of People's Republic of China (2010 edition) (Commission of Chinese Pharmacopoeia, 2010). PMR, PMRP-A and PMRP-B used in this research were shown in Fig. 2.

2.3. Extract of PMR, PMRP-A and PMRP-B

100 g powders (60 meshes) of PMR, PMRP-A and PMRP-B was refluxed with water (900 mL, 700 mL and 700 mL) for 3 times, separately. Extracts were combined, condensed and lyophilized. Water extract yields of PRM, PMRP-A and PMRP-B were 16.53%, 21.92% and 16.96%. Similarly, these powders were refluxed with 50% ethanol by the same procedure mentioned above. The 50%

Fig. 2. Photographs of Polygoni Multiflori Radix and its processed products: (A) PMR, (B) PMRP-A, (C) PMRP-B. PMR, Polygoni Multiflori Radix; PMRP-A, Polygoni Multiflori Radix Praeparata (steamed solely); PMRP-B, Polygoni Multiflori Radix Praeparata (steamed with black soybean decoction).