Journal of Ethnopharmacology 209 (2017) 140–146

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Effect of cortex mori on pharmacokinetic profiles of main isoflavonoids from *pueraria lobata* in rat plasma

Bingxin Xiao[a,b], Zengxian Sun[a], Shu yang Sun[c], Jie Dong[a], Yanli Li[a], Shan Gao[a], Jie Pang[a], Qi Chang[b,*]

[a] The First People's Hospital of Lianyungang, Lianyungang 222002, PR China
[b] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, PR China
[c] Overseas Education College, Nanjing Tech University, Nanjing 211816, PR China

ARTICLE INFO

Keywords:
Pueraria lobata
Cortex mori
Anti diabetes
Pharmacokinetic
LC-MS/MS

ABSTRACT

Ethnopharmacological relevance: Radix pueraria (the root of *pueraria lobata (Wild.) Ohwi.*), which contains a class of isoflavonoids as the main active components, as well as cortex mori (the root bark of *Morus alba L*), which contains abundant active alkaloids, have been employed for the treatment of diabetes in traditional Chinese medicine for centuries. In previous studies, pharmacodynamic synergistic reactions have been observed in compatible application of *pueraria lobata* isoflavonoids extracts (PLF) and cortex mori alkaloids extracts (CME) for inhibiting α-glycosidase activity. It has also been demonstrated that PLF can effectively slow down the absorption of active alkaloid from CME, so as to produce a higher effective concentration in small intestine for depressing the elevation of postprandial blood glucose through inhibiting α-glycosidase activity.

Aim of the study: In this study, the hypoglycemic effect of PLF, CME or CME-PLF mixture (the mixture of CME and PLF at a ratio of 1:6.3) was further evaluated through in vivo glucose tolerance studies. And the effect of CME on pharmacokinetic profiles of main isoflavonoids from PLF in rat plasma was investigated to further underlie compatibility mechanism of the two herbs.

Materials and methods: Four groups of rats received an oral dose of starch solution alone or simultaneously with drugs by gavage feeding. The blood samples were collected to determine glucose concentrations by glucose oxidase method. In addition, another two groups of rats were orally administered with PLF or CME-PLF. The plasma samples were collected and assayed using an LC/MS/MS method for comparatively pharmacokinetic studies of five main isoflavonoids.

Results: For starch loading, co-administration of CME-PLF resulted in more potent inhibition effects on glucose responses compared to those by CME or PLF in rat. The isoflavonoids from PLF were rapidly absorbed, presenting similarly low concentrations in plasma. When CME was added, the C_{max} and AUC of all the five isoflavonoids were increased. A phenomenon of double peaks was found for all analysts. The elimination rates of all the detected isoflavonoids were also slowed down with extension of $t_{1/2}$.

Conclusions: CME has been found to increase the absorption and delay the elimination of main isoflavonoids from PLF, which might result in higher concentrations of circulating active compounds for anti diabetes.

1. Introduction

Diabetes mellitus is a disease manifested with chronic hyperglycemia and multiple metabolic disturbances. The disease can lead to several complications related with cardiovascular disease, renal failure or neurological disorders, which severely impairs patients' lives (Goh and Cooper, 2008; Whiting et al., 2011). The current therapy for diabetes includes mainly single-target drugs and insulin. Continuous use of these causes undesirable side effects such as abdominal pain, flatulence, meteorism and diarrhea (Wang et al., 2016). The utilization of naturally occurring medicinal plants has caught scientists' attentions for development of safe and efficient anti-diabetic agents

Abbreviations: PU, puerarin; MPU, 3'-methoxypuerarin; HPU, 3'-hydroxypuerarin; DA, daidzein; DAC, daidzein-8-C-apiosyl-(1-6)-glycoside; PLF, pueraria lobata isoflavonoids extracts; CME, cortex mori alkaloids extracts; CME-PLF, the mixture of pueraria lobata isoflavonoids extracts and cortex mori alkaloids extracts; ICA, Icarisid II; LC-MS/MS, liquid chromatography tandem mass spectrometry

* Correspondence to: Institute of Medicinal Plant Development, Chinese Academy of Medical sciences & Peking Union Medical College, No.151, Malianwa North Road, Haidian District, Beijing 100193, PR China.
E-mail address: zm870701@163.com (Q. Chang).

http://dx.doi.org/10.1016/j.jep.2017.07.035
Received 22 January 2017; Received in revised form 10 July 2017; Accepted 21 July 2017
Available online 21 July 2017

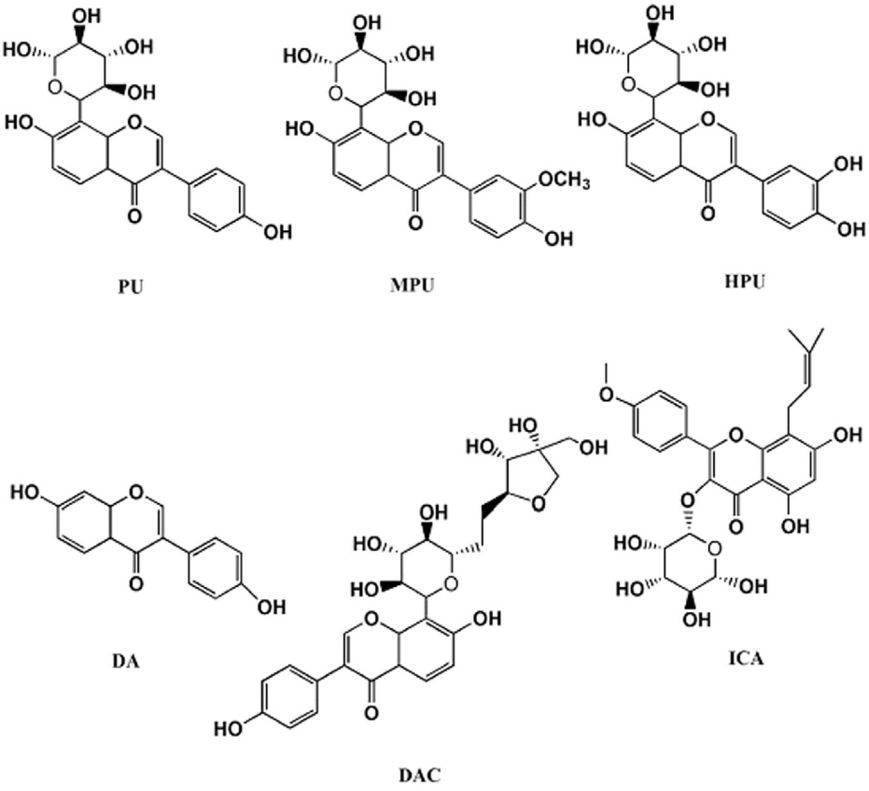

Fig. 1. Chemical structures of the analytes, puerarin (PU), 3'-methoxypuerarin (MPU), 3'-hydroxypuerarin (HPU), daidzein (DA) and daidzein-8-c-apiosyl-(1-6)-glycoside (DAC) and internal standard Icarisid II (ICA).

(Al-Malki et al., 2015; Adisakwattana et al., 2012; He et al., 2011; Kawser Hossain et al., 2016).

Radix pueraria, the root of *Pueraria lobata (Wild) Ohwi*, is suggested for the treatment of fever, acute dysentery, diarrhea, thirst, and diabetes (xiaoke zheng) in traditional Chinese medicine, with a recommended dose of 10–15 g (PPRC, 2010; Zhang et al., 2013), eg. the clinical application of Gegenqinlian decoction with radix pueraria as the main prescription for ant diabetes (Li, 2015). Isoflavonoids of radix pueraria, including puerarin (PU), 3'-methoxypuerarin (MPU), 3'-hydroxypuerarin (HPU), daidzein (DA), formononetin and their C- or O-glycosides, are considered to be the most abundant constituents and responsible for many pharmacological actions of radix pueraria. In our previous studies (Xiao et al., 2016), six main isoflavonoids of PU, DA, MPU, HPU, daidzin and daidzein-8-C-apiosyl-(1-6)-glucoside (DAC) were determined in self prepared *pueraria lobata* isoflavonoids extracts (PLF). The anti-diabetic functions of *pueraria lobata* isoflavonoids have been demonstrated by several studies (Wong et al., 2011). In an animal study, chronic dietary supplementation with the extract of *pueraria lobata* (containing ~ 23% puerarin, w/w) significantly decreased baseline fasting glucose and improved insulin sensitivity (Prasain et al., 2012). The components of PU, daidzin, MPU and DA were considered to be responsible for the effect of a diet formula containing *pueraria lobata* on enhancing glucose utilization and preventing triglycerides accumulation in mice (Liu et al., 2014). In addition, PU, the most abundant isoflavonoid of *pueraria lobata*, has been demonstrated to improve glucose tolerance and inhibit glucose uptake under both administration routes (oral and i.p.) in rat, while daidzin showed an opposite effect of stimulating glucose uptake (Elias et al., 2005). The study also showed that oral administration of PU is not as effective as i.p. injection which may be due to the much higher bioavailability in blood when given i.p. (Elias et al., 2005). The similar anti diabetic activity of orally administered PU was observed in rat in a dose dependent manner in another study (She et al., 2014). A higher bioavailability and dose of PU could produce more potent hypoglycemic effects due to the resulting higher concentration of PU in vivo. Another isoflavonoid from *pueraria lobata*, DA, has also showed favorable effects for anti-diabetes in high-fat diet-induced C57BL/6 J mice (Zang et al., 2015). Limited researches have been reported on the anti diabetic activity of other isoflavonoids from PLF. In present study, five isoflavonoids except daidzin which showed controversial hypoglycemic action have been considered as the main active compounds of PLF with determined anti diabetic activities as well as high contents.

Cortex mori, the root bark of *Morus alba* L., has been used in traditional chinese medicine as an diuretic and expectorant agent (PPRC, 2010), with a recommended dose of 6–12 g. It was first recorded for anti-diabetes in “Compendium of Materia edica”. As our previous studies showed, PLF could effectively slow down the oral absorption of main active component of cortex mori alkaloids extracts (CME) with the decrease of C_{max} and delay of T_{max}, resulting in a relatively stronger inhibition effect on α-glycosidase in small intestine and more potent effect for depressing the elevation of postprandial blood glucose (Xiao et al., 2014). In present study, the effect of CME on pharmacokinetic profiles of five main active isoflavonoids, PU, MPU, HPU, DA and DAC from PLF were determined to see if higher circulation concentrations of active compounds from PLF were achieved through the compatibility of CME to further elucidate the increasing efficacy of CME-PLF mixture.

2. Material and methods

2.1. Reagents and chemicals

The commercial herbs of cortex mori (YlOl113HB) and pueraria lobata (G110113AH) were provided by Beijing Peking University, WBL Biotech Co. Ltd. For quality control, the fingerprint of total isoflavonoids from pueraria lobata and total alkaloids from cortex mori has been determined by our previous studies (Duan et al., 2013). The extracts of PLF (PU, 279 mg/g; MPU, 62.3 mg/g; HPU, 57.8 mg/g; DA,