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REVIEW

Puerarin: A Review of Pharmacological Effects

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Puerarin is the major bioactive ingredient isolated from the root of the *Pueraria lobata* (Willd.) Ohwi, which is well known as Gegen (Chinese name) in traditional Chinese medicine. As the most abundant secondary metabolite, puerarin was isolated from Gegen in the late 1950s. Since then, its pharmacological properties have been extensively investigated. It is available in common foods and is used in alternative medicine. It has been widely used in the treatment of cardiovascular and cerebrovascular diseases, diabetes and diabetic complications, osteonecrosis, Parkinson's disease, Alzheimer's disease, endometriosis, and cancer. The beneficial effects of puerarin on the various medicinal purposes may be due to its wide spectrum of pharmacological properties such as vasodilation, cardioprotection, neuroprotection, antioxidant, anticancer, antiinflammation, alleviating pain, promoting bone formation, inhibiting alcohol intake, and attenuating insulin resistance. However, the direct molecular mechanisms and targets remain unclear. This review provides a comprehensive summary of the pharmacological effects of puerarin. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: puerarin; *Pueraria lobata*; pharmacology.

INTRODUCTION

Puerarin is the major bioactive ingredient derived from the root of the *Pueraria lobata* (Willd.) Ohwi, widely known as Gegen (Chinese name) in traditional Chinese medicine (Chen *et al*., 2012c). Gegen is native to Southeast Asia and has been employed as a food source, medicine, and fodder for thousands of years and is one of the earliest medicinal herbs utilized in ancient China (Prasain *et al*., 2003). It is used frequently to treat fever, diarrhea, emesis, cardiac dysfunctions, liver injury, weight loss, and toxicosis (Wong *et al*., 2011).

As one of three major isoflavonoid compounds, puerarin (chemical structure is shown in Fig. 1) was isolated from Gegen in the late 1950s. Since then, its pharmacological properties have been extensively investigated (Rong *et al*., 2002). It has been widely used in the treatment of cardiovascular diseases, cerebrovascular disorders, cancer, Parkinson's disease (PD), Alzheimer's disease (AD), and diabetes and diabetic complications. It also exerts protective actions against fever, inflammation, hyperlipidemia, osteonecrosis, alcohol-induced disorders, and oxidative damage. The beneficial effects of puerarin on various medicinal purposes may be due to its abilities to inhibit calcium influx, improve microcirculation, reduce insulin resistance, scavenge oxygen free radicals, counteract cell death, inhibit alcohol intake, and so on. Currently, there are three main dosage forms of puerarin for clinical applications, that is, injection, tablet, and capsule (Wang *et al*., 2006). The form of injection has been approved by the State Food and Drug Administration in China (Hou *et al*., 2011).

Over the past decades, there has been a large amount of research on the pharmacological effects of puerarin. The aim of this review is to give a comprehensive summary and analysis to its pharmacological properties.

PHARMACOLOGICAL ACTIONS

Vasodilatory activity

Recently, multiple reports have confirmed the vasodilatory action of puerarin on rat aorta. Puerarin exerted the vasodilatory effect on rat thoracic aortas by activating large-conductance voltage-activated and calcium-activated potassium (BKCa) channels that act as a significant part in the modulation of vascular tone (Sun *et al*., 2007). Activation of BKCa channels results in hyperpolarization of cell membrane, which conducts voltage-dependent calcium channels devitalization, followed by vasodilation (Brenner *et al*., 2000).

Puerarin (50, 150, and 450 μM) could concentration-dependently relax the endothelium-intact rat aortic rings preconstricted with phenylephrine (10^{-9} to 10^{-6} M) or KCl (10–100 mM). However, the relaxant effect was not observed in the endothelium-denuded rat aortic rings, indicating that the vasorelaxant effect conducted by puerarin was dependent on the activation of endothelium. Furthermore, the antivasoconstrictive effect of puerarin in response to phenylephrine was completely abrogated in Ca^{2+}-free solution. The antivasoconstrictive effect of puerarin was evidently attenuated by 100 μM of

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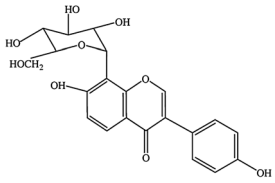

Figure 1. The chemical structure of puerarin.

N^G-nitro-L-arginine methyl ester [L-NAME, an inhibitor of nitric oxide synthase (NOS)], 10 μM of indomethacin (an inhibitor of cyclooxygenase), and 1 μM of 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (an inhibitor of NO-sensitive soluble guanylylcyclase) as well as the three kinds of K^+ channel inhibitors, glibenclamide (3 μM, a specific inhibitor of adenosine triphosphate (ATP)-sensitive K^+ channel), tetraethylammonium (10 mM, a specific inhibitor of K_{Ca}^{2+}), and Ba^{2+} (0.25 mM, a specific inhibitor of K_{IR}), but obviously reinforced by 8-bromo-guanosine 3′-5′-cyclic monophosphate (100 μM, an analogue of cGMP). Additionally, puerarin (10–160 μM) decreased NO production in rat aortic cells in a concentration-dependent manner. These results indicate that the endothelium-dependent antivasoconstrictive effect of puerarin is relevant to triggering extracellular Ca^{2+} influx into endothelial cytosol, which involves the endothelial Ca^{2+}–NO–cGMP pathway, prostacyclin, and opening of the three K^+ channels (Yan *et al*., 2009).

However, the relaxant effect of puerarin on porcine aortic rings is considered endothelium independent. Puerarin (10 μM) strengthened the relaxation mediated by endothelium-independent vasodilators, sodium nitroprusside (SNP), and cromakalim. L-NAME and Triton X-100 had no effect on the strengthening action of puerarin on SNP-induced vasodilation. Additionally, the relaxation strengthened by puerarin was reversible following a washout procedure, suggesting that puerarin exerts the enhancement of vasodilation via non-genomic signaling cascade. Moreover, the 3′-5′-cyclic adenosine monophosphate (cAMP) pathway was involved in the potentiating effect of puerarin on endothelium-independent vasodilation (Yeung *et al*., 2006).

Puerarin also had a cerebral vasodilatory effect on rat basilar artery rings precontracted with U46619 (100 nM), which brought out 50% inhibition (IC_{50}) of 304 ± 49 μM. The cerebral vasodilation mediated by puerarin was associated with both endothelium-dependent and endothelium-independent pathways. The endothelium-dependent vasodilation induced by puerarin was related to NO production, whereas the endothelium-independent relaxation involved the K^+ channels opening (Deng *et al*., 2012).

Cardioprotective activity

Puerarin exerted the cardioprotective effect via sodium and L-type calcium channels in guinea-pig and rat ventricular myocytes (Zhang *et al*., 2003; Guo *et al*., 2004). The cardioprotective effect of puerarin against ischemia and reperfusion injury in isolated ventricular myocytes was carried out through activation of mitochondrial ATP-sensitive potassium channels and inhibition of the opening of mitochondrial permeability transition pores (Gao *et al*., 2006). The production of myocardial formazan augmented and the release of lactate dehydrogenase diminished during reperfusion when the isolated rat heart was administered puerarin (0.24 mmol/L) before ischemia. However, this action was counteracted by treatment with 1 μmol/L of paxilline (a blocker of the calcium-activated potassium channel). Besides, puerarin attenuated hydrogen peroxide (H_2O_2)-elicited myocyte death and decreased the level of reactive oxygen species (ROS) in isolated ventricular myocytes. Nevertheless, these alterations were reversed by both chelerythrine [an inhibitor of protein kinase C (PKC)] and paxilline (Yang *et al*., 2008), suggesting that the cardioprotective activity of puerarin against ischemia and reperfusion injury is associated with the opening of calcium-activated potassium channels and activation of PKC (Gao *et al*., 2007).

Puerarin could recover the increased mRNA expression of cardiac transforming growth factor β1 (TGF-β1) and SMAD3 and reduced mRNA expression of cardiac SMAD7 in spontaneous hypertensive rats. These alterations in gene expression subsequently ameliorated myocardial hypertrophy and fibrosis, which might be a mechanism underlying the myocardial protective property of puerarin from hypertension (Zhang *et al*., 2011c). In addition, puerarin lowered the mRNA and protein expression levels of apelin and its receptor APJ in the left ventricle tissue of two-kidney, one-clip renal hypertension rats (Jin *et al*., 2009).

Intraperitoneal injection of puerarin (100 mg/kg) effectively alleviated myocardial ultrastructural damages in streptozotocin (STZ)-induced diabetic rats. Moreover, the expression of thrombospondin-1 (TSP-1) in myocardium was lessened, whereas the left ventricular developed pressure and left ventricular systolic end pressure were elevated. These data suggest that puerarin elicits the myocardial protective effect by blocking TSP-1 expression, followed by amelioration of the left ventricular function in diabetic rats (Pan *et al*., 2009). Intraperitoneal injection of puerarin (120 mg/kg) up-regulated myocardial endothelial nitric oxide synthase (eNOS) gene and protein expression and protein kinase B (Akt/PKB) phosphorylation and subsequently increased serum NO production in rats with myocardial infarction, which might be the possible mechanism underlying the therapeutic action of puerarin in coronary artery diseases (Zhang *et al*., 2008d).

The protective effect of puerarin on myocardial fibrosis was evaluated using isoprenaline (ISO)-induced myocardial fibrotic mouse model. The situation of myocardal fibrosis effectively ameliorated after treatment with puerarin (600 and 1200 mg/kg) for 40 days. Moreover, puerarin notably reduced collagen volume fraction, collagen accumulation, hydroxyproline content, and cardiac weight index in myocardial tissue. Puerarin lowered the mRNA and protein expression levels of TGF-β1 and the protein expression level of nuclear factor-kappa B (NF-κB) and significantly up-regulated the mRNA expression of peroxisome proliferator-activated receptor (PPAR) α/γ in myocardial tissue, indicating that puerarin ameliorates myocardial fibrosis induced by ISO in mice by down-regulation of TGF-β1 expression, activation of PPAR α/γ, and subsequently suppression of NF-κB in myocardial tissue (Chen and Chan, 2009).

Puerarin could alleviate myocardial ischemia injury in dogs with acute myocardial infarction (AMI) induced by

ligation of coronary artery through reduction of the infarct zone and increase of collateral vessels, capillaries, and distribution vessel density in ischemic area. The increased blood viscosity and platelet aggregation during AMI were alleviated by puerarin treatment. These changes resulted in improvement of microcirculation and restriction of myocardial infarct area (Liu *et al*., 2000).

Intravenous administration of puerarin apparently alleviated the symptoms in patients with infantile viral myocarditis by augmentation of myocardium metabolism and amelioration of the cardiac function, indicating that puerarin might act as an effective drug for infantile viral myocarditis (Meng *et al*., 1999).

Inhibition of ischemia and reperfusion injury

Intraperitoneal pretreatment with puerarin (25 and 50 mg/kg) dose-dependently alleviated focal cerebral ischemia induced by middle cerebral artery occlusion (MCAO) in rats. Puerarin significantly inhibited brain infarct size and increased hypoxia-inducible factor-1α, inducible nitric oxide synthase (iNOS), TNF-α, and active caspase-3 protein expression levels in MCAO-stimulated ischemic area. Administration of puerarin (50 and 100 mg/kg) decreased edema volume, and glutamate (Glu) and aspartate (Asp) levels but enhanced neurological function (Xu *et al*., 2007). Furthermore, puerarin decreased NF-κB translocation (Ding *et al*., 2007), neurological deficit, and apoptosis of neurocytes but elevated p-Akt (Ser473) expression in cerebral ischemia/reperfusion rats. Nevertheless, these alterations were reversed by LY294002 (a specific inhibitor of P13K kinase), suggesting that puerarin might attenuate apoptosis of neurons and prevent rats from cerebral ischemia/reperfusion injury via activation of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway (Han *et al*., 2012). Puerarin (10–50 μM) concentration-dependently inhibited respiratory bursts induced by formyl-Met-Leu-Phe in human neutrophils (Chang *et al*., 2009). Consequently, puerarin might be a potent neuroprotective agent against disorders associated with ischemia–reperfusion brain injury.

By a blockage of acid-sensing ion channels, puerarin suppressed hippocampal cell death induced by acidosis, a byproduct of brain ischemia, indicating the neuroprotective mechanism of puerarin against brain ischemia (Gu *et al*., 2010). Puerarin could abrogate *N*-methyl-D-aspartate receptor expression within 12 h after cerebral ischemia in rat hippocampus CA1 region, suggesting that puerarin prevents from neural injury by counteraction of the toxic effect of excitatory amino acids (Zhang *et al*., 2011d). Administration of puerarin (10 and 40 μM) markedly inhibited apoptosis and necrosis induced by 3-h oxygen/glucose deprivation (OGD) in the cultured hippocampal neurons of Sprague-Dawley rats and significantly attenuated OGD-stimulated Ca^{2+} influx, intracellular Ca^{2+} peak, and NO synthesis. Puerarin also reduced apoptosis and necrosis induced by glutamate (Glu, 0.5 mM), suggesting that the neuroprotective property of puerarin against cerebral ischemia is related to the suppression of the intracellular Ca^{2+} level, neuronal NO synthesis, and apoptosis cascade activation (Xu and Zheng, 2007). In another study, rats were subjected to 2 h of MCAO, followed by up to 72 h of reperfusion. Intraperitoneal injection of puerarin (200 and 400 mg/kg) reduced infarction volume and ameliorated functional neurological outcome. In addition, puerarin limited apoptosis and up-regulated erythropoietin expression, indicating that the neuroprotection of puerarin against ischemic brain injury is due in part to stimulation of erythropoietin activation and attenuation of apoptosis (Gao *et al*., 2009a).

Intraperitoneal injection of puerarin (50 mg/kg) elevated the mRNA expression of thioredoxin-1/2 and down-regulated the apoptosis index in rats with spinal ischemia–reperfusion injury (Tian *et al*., 2011). Moreover, puerarin could accelerate spinal blood flow (Zhou *et al*., 2006) and attenuate the spinal cord damage in ischemia–reperfusion rabbit models, suggesting the neuroprotective effect of puerarin against spinal ischemia–reperfusion injury. The neuroprotective effect of puerarin on the injured brain neurocytes has been confirmed using acute local cerebral ischemia and ischemia–reperfusion rat models induced by ligating the middle cerebral artery, showing that puerarin improves acute cerebral ischemia and ischemia–reperfusion injuries by elevation of HSP70 expression and reduction of the Fas expression (Pan and Li, 2008).

Puerarin ameliorated the learning–memory disorder induced by global cerebral ischemia–reperfusion injury in rats by inhibiting or delaying apoptosis after cerebral ischemia–reperfusion related to the increase of Bcl-2 proteins (Wu *et al*., 2009). Puerarin also elevated the ability of learning–memory in D-galactose-stimulated senile mice and reperfusion mice with bilateral carotid arteries occlusion (Xu and Zhang, 2002).

The clinical study demonstrated that intravenous drip of puerarin (2 mg/mL) down-regulated the thromboxane B2 level and cerebral vasospasm (CVS) incidence in patients with aneurysm subarachnoid hemorrhage (aSAH). Moreover, puerarin up-regulated the levels of NO, 6-keto-prostaglandin F1 alpha, and endothelin-1 in plasma, the mean middle cerebral artery velocity, and the Glasgow outcome scale at discharge, indicating that puerarin might be an effective therapeutic medication against CVS in patients with aSAH by modulation of vascular active factors levels, facilitation of cerebral blood flow, and amelioration of cerebral perfusion (Wang *et al*., 2012a).

Antidiabetic activity and inhibition of diabetic complications

Antidiabetes. Puerarin has been used for the therapy of diabetes mellitus (DM) in China since the 1990s. Intravenous administration of puerarin dose-dependently lowered the blood glucose level (Hsu *et al*., 2003), up-regulated the mRNA and protein expression of glucose transporter-4 in soleus muscle, and evoked α1-adrenoceptors in the adrenal gland to increase β-endorphin secretion, leading to down-regulation of plasma glucose content in STZ-induced diabetic rats (Xie and Du, 2011). In male Sprague-Dawley rats with high-fat diet, the augmented body weight gain and impaired glucose/insulin resistance were recovered, the increased serum resistin and leptin levels were evidently blocked, and the inhibited mRNA expression of leptin and resistin in epididymal white adipose tissue was enhanced when treated with puerarin (100 and 200 mg/kg) (Zhang et al., 2010a, 2010b). Puerarin (100 μM) markedly

suppressed H_2O_2-induced viability loss and apoptotic index in pancreatic islets. Additionally, pretreatment with puerarin improved H_2O_2-induced reduction in basal and glucose-stimulated insulin production but blocked H_2O_2-induced free radicals production and stimulated activities of superoxide dismutase (SOD) and catalase in the isolated pancreatic islets, suggesting the protective effect of puerarin on pancreatic islets against oxidative stress associated with the antioxidative activity (Xiong *et al*., 2006). Thus, puerarin might play an important role in preventing islet cells from oxidative stress in DM and could be a novel therapeutic drug against DM.

Puerarin significantly enhanced glucose uptake into the insulin sensitive cell (Kato and Kawabata, 2010) and lowered blood glucose levels, triglycerides, insulin, total cholesterol content, and body weight in obese Zucker rats and genetic rats with type 2 DM (T2DM; Zhu *et al*., 2010b). Glucose and insulin metabolism were ameliorated by a kudzu diet including puerarin in C57BL/6J ob/ob mice (Prasain *et al*., 2012). Puerarin obviously blocked the mRNA expression of adipose differentiation-related protein (ADRP) gene in fatty tissue of T2DM rats (Sun *et al*., 2008). Furthermore, puerarin could markedly relieve insulin resistance, the principal problem in T2DM, induced by free fatty acid in 3T3-L1 lipocytes. Particularly, puerarin evidently elevated the mRNA expression of PPAR-γ and Cb1 binding protein, membranous distribution of glucose transporter-4 as well as glucose transportation in 3T3-L1 lipocytes (Zhao and Zhou, 2012), up-regulated 3T3-L1 preadipocytes differentiation, glucose-6-phosphate dehydrogenase (G6PDH) activity, lipid accumulation, and the mRNA expression of G6PDH, catalase, glutathione reductase, and adiponectin, resulting in an inhibition of insulin resistance and diabetic states (Lee *et al*., 2010).

Puerarin could elevate eNOS phosphorylation on Ser1177, leading to an up-regulation of NO production in EA.hy926 cells. The enhancement of eNOS phosphorylation was associated with the activation of PI3K/Akt dominated by estrogen receptor (ER) and AMP-activated protein kinase (AMPK) mediated by calcium/calmodulin-dependent kinase II (CaMKII). Moreover, puerarin suppressed the expression of intercellular cell adhesion molecule-1 (ICAM-1) and the activation of NF-κB induced by TNF-α, and prevented the adhesion of TNF-α-stimulated monocytes to endothelial cells. In conclusion, puerarin enhanced eNOS phosphorylation and NO production related to the ER-dependent PI3K/Akt and CaMKII-mediated AMPK pathway, which might contribute to the beneficial effect of puerarin against endothelial dysfunction associated with T2DM (Hwang *et al*., 2011).

Inhibition of diabetic ocular complications. Puerarin could markedly ameliorate STZ-induced diabetic retinopathy in rats, attenuate the increased Fas/FasL expression induced by $ONOO^-$ in retinal pigment epithelial (RPE) cells (Hao *et al*., 2011b), inhibit the mRNA and protein expression of iNOS and activation of C3, prevent $ONOO^-$-induced apoptosis, and reduce nitrotyrosine (NT) and $ONOO^-$-mediated protein nitration production in RPE cells (Hao *et al*., 2010). Puerarin protected RPE cells against apoptosis in diabetic rats by repression of iNOS expression and peroxynitrite level. Intraperitoneal injection of puerarin (140 mg/kg) retarded the mRNA or protein expression of Fas/FasL, NT, iNOS, and apoptosis of RPE cells in diabetic rats induced by STZ injection (45 mg/kg) (Hao *et al*., 2012). Puerarin (500 mg/kg) treatment enhanced SOD activity, lowered malondialdehyde (MDA) content in serum and retinas of early diabetic rats induced by STZ, and inhibited the mRNA and protein expression of advanced glycation end-product (AGE) receptor and vascular endothelial growth factor in retinas (Chen *et al*., 2012b). Moreover, the increased mRNA expression of hypoxia-inducible factor-1α in retina of STZ-stimulated diabetic rats was notably suppressed by puerarin administration (Teng *et al*., 2009).

Marked or complete opacities in the lens were observed in STZ-treated rats, whereas only slight lens opacities occurred in rats with peritoneal and peribulbar injection of puerarin. STZ lessened lens epithelial cell (LEC) volume with a dense nucleus, induced many bubbles around the equator area, and stimulated the elevation of cell gap, unclear structure of rough endoplasmic reticulum, the abnormality of fibers, and mitochondria swelling in LEC. Up-regulated NO, NOS, iNOS protein, and mRNA expressions were observed in the lens of STZ-treated rats. Additionally, STZ enhanced the expressions of NT and Fas/FasL and caused the normal gene DNA ladder to typical 'ladder bands' (Hao *et al*., 2008). However, these changes were minor in puerarin-treated rats, suggesting that puerarin might be beneficial to the treatment of rat diabetic cataract (Hao *et al*., 2011a). Puerarin attenuated the retinal pericytes apoptosis induced by advanced AGE-modified bovine serum albumin (BSA) *in vitro* through prevention of p47phox-dependent and Rac1-dependent NADPH oxidase activation, ROS generation, and NF-kB activation (Kim *et al*., 2012).

Inhibition of diabetic vascular complications. Puerarin could inhibit the proliferation of rat vascular smooth muscle cells (VSMC) induced by high glucose (HG) *in vitro* and *in vivo* through suppression of PKC β2/Rac1-dependent signaling pathway. In detail, puerarin markedly attenuated neointimal formation of obese Zucker rats induced by balloon injury *in vivo* and abolished HG-induced phosphorylation and membrane translocation of PKCβ2, leading to prevention of phosphorylation and membrane translocation of Rac1, p47phox, and p67phox subunits as well as NADPH oxidase activation and ROS production in VSMCs *in vitro* (Zhu *et al*., 2010b). Puerarin dose-dependently suppressed HG-induced acute vasoconstriction and vasodilation dysfunction, whereas it enhanced heme oxygenase (HO) activity and HO-1 protein expression in rat thoracic aorta. On the other hand, this protective effects were reversed by ZnPP (a blocker of HO-1), indicating that puerarin alleviated HG-induced vascular dysfunction in rat aortic rings by increasing HO-1 activity (Meng *et al*., 2009). Furthermore, puerarin inhibited HG-induced decrease of vascular contraction responses to phenylephrine in rat aortic rings because of activation of HO-1 (Zhu *et al*., 2011). In addition, puerarin lowered the levels of blood P-selectin, low density lipoprotein (LDL), and cholesterol as well as the mRNA expression of aorta vascular cell adhesion molecule in rats with STZ-induced diabetes (Li *et al*., 2007). These results demonstrated the protective effect of puerarin against vascular complications in DM.

Amelioration of diabetic nephropathy. In STZ-induced diabetic nephropathy rat model, puerarin lowered the levels of urinary albumin excretion, serum creatinine and blood urea nitrogen but up-regulated the heparan sulfate proteoglycan expression and creatinine clearance rate (Li *et al*., 2009). Puerarin suppressed renal AGEs formation via reduction of blood glucose levels, aldose reductase activity, and oxidative stress, and also lowered the mRNA expression of AGE-specific cellular receptor in renal cortex of STZ-induced diabetic rats (Shen *et al*., 2009). Furthermore, puerarin decreased the serum level of collagen type IV in STZ-induced diabetic nephropathy rats (Li *et al*., 2008a) and attenuated PKC activity, the accumulation of glomerulus extracellular matrix, and the levels of c-jun, c-fos, and collagen type IV in glomerular mesangial cells cultured in HG medium (Mao and Gu, 2005). Puerarin might be a novel therapeutic agent against STZ-induced diabetic nephropathy.

Antiinflammatory activity

The antiinflammatory property of puerarin was verified in a mouse ear edema model (Lee *et al*., 1994) and in several cellular models of inflammation. Puerarin dose-dependently suppressed the phosphorylation and degradation of inhibitor-κB, and translocation of nuclear NF-κB p65 subunit (Xie and Du, 2011), resulting in the suppression of the protein and mRNA expression of C-reactive protein (CRP) in lipopolysaccharides (LPS)-stimulated peripheral blood mononuclear cells (PBMCs; Yang *et al*., 2010). Puerarin suppressed the expression of CRP, iNOS, and cyclooxygenase-2, resulting in the inhibition of the NF-κB signaling pathway in LPS-stimulated RAW264.7 cells (Hu *et al*., 2011) and markedly depressed LPS (1 mg/L)-induced morphological changes of RAW264.7 cells. Moreover, the mRNA expression of NF-κB p65 in cells and the levels of macrophage inflammatory protein 2 and TNF-α in cell supernatant were inhibited by pretreatment with puerarin (Hu *et al*., 2012). Palmitate activated IKKβ/NF-κB and increased the expression and production of TNF-α and interleukin-6 (IL-6) in endothelial cells, and these effects were effectively reversed by treatment with puerarin. Furthermore, puerarin attenuated serine/tyrosine phosphorylation of insulin receptor substrate-1 and ameliorated the impaired insulin PI3K signaling pathway induced by palmitate, resulting in up-regulation of insulin-mediated NO production from the endothelium (Huang *et al*., 2012a).

Puerarin inhibited AGE-stimulated inflammation in mouse mesangial cells by up-regulation of HO-1 expression, which was attenuated by the reduction of endogenous PKCδ with specific small interfering RNA (siRNA), GF109203X (a blocker of PKC), and rottlerin (a specific blocker of PKCδ), but not Gö6976 (a selective inhibitor of PKC α/β II). In addition, puerarin enhanced the activity of antioxidant response element-luciferase and translocation of NF-E2 related factor-2 (Nrf2), resulting in an increase of HO-1 expression. Pretreatment with puerarin prevented the expression of cyclooxygenase-2, matrix metallopeptidase 2 (MMP-2), and MMP-9, which was reversed by ZnPP. Puerarin induced HO-1 expression via the PKC δ-Nrf2-HO-1 pathway, thereby exerting its property of antiinflammation (Kim *et al*., 2010).

Puerarin notably attenuated the mRNA expression of IL-8 in human bronchial epithelial (BEAS-2B) cells and release of IL-8 in the culture supernatant of the co-culture of BEAS-2B cells and human neutrophils, suggesting that puerarin exhibited the antiinflammatory activity by inhibiting the level of IL-8 (Pang *et al*., 2012). Puerarin treatment ameliorated the pathological situation and facilitated metabolic function in the liver of CCl4 (2 mL/kg)-induced hepatic fibrosis (HF) rats, resulting in down-regulation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, and albumin. Meanwhile, puerarin suppressed the protein or mRNA expression of TNF-α, NF-κB, and iNOS, and the production of TGF-βl and MDA, but enhanced SOD activity (Li *et al*., 2013). The protective mechanism of puerarin against CCl4-induced hepatotoxicity in HF rats was associated with the improvement of metabolic function and antiinflammation response in liver tissue.

Anti-Parkinson's disease activity

Puerarin was evaluated for its protective effect against dopamine neuron loss by using the 1-methyl-4-phenylpyridinium (MPP^+)-insults dopaminergic SH-SY5Y cellular model, a typical PD model. Puerarin pretreatment enhanced Akt phosphorylation in both MPP^+-induced and non-MPP^+-induced cells, modulated mitochondrial membrane potential, and prevented MPP^+-induced apoptosis, which were effectively abolished by LY294002 (Cheng *et al*., 2011; Zhu *et al*., 2012). Moreover, puerarin suppressed MPP^+-induced p53 nuclear accumulation, and Puma and Bax expression, subsequently causing caspase-3-dependent programmed cell death (PCD), which were reversed by the PI3K/Akt inhibitor as well as Pifithrin-α (an inhibitor of p53). Therefore, the PI3K/Akt signaling pathway was relevant to the protective property of puerarin against MPP^+-induced neuronal loss by blocking p53-mediated and caspase-3-dependent PCD (Zhu *et al*., 2012). Puerarin could protect MPP^+-induced SH-SY5Y cells from apoptosis through mediation of the ubiquitin proteasome system function. Puerarin enhanced protease activity in MPP^+-induced cells, leading to a decrease of ubiquitin-conjugated proteins accumulation (Cheng *et al*., 2009). Intraperitoneal injection of puerarin (0.12 mg/kg) protected dopaminergic neurons of the substantia nigra against 6-hydroxydopamine-induced cell death through suppression of apoptosis and elevation of glial cell line-derived neurotrophic factor expression in rats with PD (Zhu *et al*., 2010a).

The viability of rat pheochromocytoma PC12 cells was obviously suppressed when they were exposed to 500 μM of MPP^+, which could be reversed by puerarin treatment in a dose-dependent manner. Puerarin also inhibited the phosphorylation of c-Jun, c-Jun-NH2-terminal kinase (JNK), and MKK7, and attenuated the release of mitochondrial cytochrome c and activation of caspase-9 and caspase-3 in MPP^+-treated PC12 cells (Bo *et al*., 2005; Wang *et al*., 2011b). SP600125 (a blocker of JNK) inhibited the protective property of puerarin against MPP^+-exposed neurotoxicity, suggesting that the neuroprotective effect of puerarin on PC12 cells is related to the inhibition of the mitochondria-dependent caspase cascade (Bo *et al*., 2005) and JNK signaling pathway. Apoptosis is a main pathogenic factor related to the development of PD; thus, puerarin might be a potential anti-PD medication.

The amount of the cells positive for tyrosine hydroxylase in the substantia nigra was less and the amount of apoptotic cells was more in PD mice receiving ovariosteresis than in the control mice, in which there were no notable differences between the puerarin-treated group and the control group. These results suggest the protective effect of puerarin, consistent with estrogen, on the nigral neurons against PD (Li *et al*., 2003). The characteristics of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model are behavioral deficits, dopaminergic neuronal degeneration, and dopamine depletion. MPTP injection progressively augmented ROS formation and evoked lysosome-associated membrane protein type 2A (Lamp 2A) expression in mice, which were reversed by puerarin administration through elevation of glial cell line-derived neurotrophic factor expression as well as activation of glutathione (GSH) and the PI3K/Akt pathway (Zhu *et al*., 2013).

Anti-Alzheimer's disease activity

Pretreatment with puerarin inhibited cell viability loss and apoptosis (Zhang *et al*., 2008a, 2008b), enhanced the expression of P-Akt, p-Bad, and Bcl-2/Bax ratio, and decreased caspase-3 activation and cytochrome c content in PC12 cells exposed to Aβ(25–35), all of which were abolished by wortmannin (a blocker of PI3K phosphorylation), demonstrating that the neuroprotective effect of puerarin against Aβ-induced neurotoxicity is related to suppression of apoptosis (Xing *et al*., 2011). Moreover, pretreatment with puerarin effectively blocked Aβ(25–35)-induced ROS overproduction and lipid peroxidation, evoked nuclear Nrf2 protein expression, and elevated the activities of catalase and GSH peroxidase and the transcription and translation levels of HO-1 in rat primary hippocampal neurons (Lin *et al*., 2012). Puerarin stimulated Serine 9 phosphorylation of GSK-3β, which was inhibited by lithium chloride (a chemical inhibitor of GSK-3β) (Zou *et al*., 2013). These findings suggest that puerarin blocks Aβ-stimulated oxidative stress via the GSK-3β/Nrf2 signaling pathway and might be a potential therapeutic candidate for AD. Injection of Aβ(1–42) induced spatial memory impairment, neuronal cell apoptosis, and caspase-9 activation in the hippocampus of rats. However, these changes were reversed after administration of puerarin, which was related to Akt activation and Bad phosphorylation, suggesting the potential use of puerarin on AD-associated neuroapoptosis and disorders of cognitive function (Li *et al*., 2010).

The preventive property of puerarin against oxidative-stress-induced neurodegeneration had been verified using mitochondrial transgenic neuronal cell cybrid models of sporadic AD (SAD). ROS accumulation, cell apoptosis, and activation of the caspase-3, p38, and JNK as well as Bax/Bcl-2 ratio were increased in SAD cybrids compared with control cybrids, which were blocked by puerarin administration. These results show that the expression of mitochondrial genes in SAD cybrids attenuates viability via the oxidative-stress-related signaling pathway and puerarin inhibits oxidative-stress-induced apoptosis by reduction of Bax/Bcl-2 ratio. Thus, puerarin might act as a scavenger of intracellular ROS and protect neurons against apoptosis stimulated by oxidative stress (Zhang *et al*., 2011a).

Antiosteoporotic activity

Puerarin in collagen matrix produced 554% more new bone than the collagen matrix alone in the parietal bone defects of New Zealand White rabbits, showing that puerarin promotes new bone formation and might be a potential medication for bone induction, bone grafting, and bone defect repair (Wong and Rabie, 2003). Puerarin (40 and 80 μMol/L) enhanced cell viability, alkaline phosphotase (ALP) activity, and mineral nodules in calvarial osteoblasts, increased the rate of bone formation in the osteoblast implants, and stimulated Akt activation in rats (Zhang *et al*., 2012b). Interestingly, all of these alterations were blocked by LY294002, suggesting that the promotive effects of puerarin on osteoblast proliferation and differentiation as well as new bone formation were modulated by the PI3K/Akt pathway (Zhang *et al*., 2007).

In ovariectomized (ovx) rats, puerarin administration enhanced ALP activity, mineralized nodules formation, collagen type I secretion, and the levels of phospho-p38 mitogen-activated protein kinase (MAPK) and β-catenin proteins in primary osteoblasts, which were blocked by ICI182780. Furthermore, puerarin ameliorated the reduced bone mineral density and content as well as femur trabecular bone structure. Therefore, puerarin exerted its ability to induce osteoblasts differentiation and bone formation via ER, p38 MAPK, and Wnt/β-catenin pathways (Wang *et al*., 2012b). In STZ-induced diabetic rats, the lowered bone mineral density, osteoblast numbers in the cortical bone, new bone formation, and the elevated caspase-3 expression were reversed by puerarin (100 mg/kg) injection, demonstrating that puerarin might be used in the treatment of diabetic osteoporosis due to the amelioration of bone metabolism affected by HG levels and the down-regulation of caspase-3 expression in osteoblasts (Liang *et al*., 2012).

The urinary deoxypyridinoline, a representative product of bone degradation, was lessened by a puerarin diet (5 mg/kg) in ovx mice. Additionally, puerarin attenuated serum tartarate-resistant acid phosphatase (TRAP) activity and alleviated the femur structure but did not improve E_2-induced uterine atrophy in ovx mice. Puerarin did not augment the growth of ER-positive human breast cancer MCF-70 cells, demonstrating that the antiosteoprotic effect of puerarin is independent of ER (Michihara *et al*., 2012).

Puerarin-loaded titanium surfaces elevated pre-osteoblasts differentiation in MC3T3-E1 cells (Yang *et al*., 2012). Puerarin could increase the amount of calcium nodules in umbilical cord mesenchymal stem cells (MSCs), accelerate differentiation of MSCs into osteoblasts, and promote MSC proliferation (Cai *et al*., 2011). Moreover, puerarin (10^{-8} mol/L) activated ALP activity, augmented osteoblast cells proliferation and matrix mineralization, and promoted NO and bone morphogenetic protein-2 (BMP-2) synthesis, leading to the increase of the gene expression of SMAD4, core binding factor α1/runt-related transcription factor 2, osteoprotegerin, and receptor activator of NF-κB ligand in imprinting control region mouse osteoblasts. Interestingly, these alterations were blocked by Noggin (an inhibitor of BMP) or L-NAME, indicating that the

osteogenic mechanism of puerarin might be related to activation of NO and BMP-2 (Sheu *et al*., 2012).

Puerarin notably enhanced the mRNA expression of osteoprotegerin and ALP, whereas it impeded the mRNA expression of receptor activator of NF-κB ligand (an osteoclastogenic factor) in rat osteoblast-like UMR106 cells. Intriguingly, ICI182780 (an antagonist of ER) could totally abrogate puerarin-stimulated increase of ALP mRNA expression. It could be summarized that puerarin stimulated bone gain through promoting osteoblast differentiation in an ER-mediated manner and might be a preventive and therapeutic candidate against postmenopausal osteoporosis (Tiyasatkulkovit *et al*., 2012).

Analgesic and antipyretic effects

Puerarin markedly enhanced mechanical withdrawal threshold and thermal withdrawal latency in superficial second paw burn rats and notably down-regulated P2X3 protein and mRNA expression in dorsal root ganglion neurons in superficial second-degree back burn rats. Puerarin might antagonize the sensitization of P2X3 receptor involved in hyperalgesia after burn injury in rats (Xu *et al*., 2009). The P2X3 protein and mRNA expression and the visual analogue scale were markedly decreased after puerarin treatment in PBMCs of burn patients (Li *et al*., 2011), up-regulated IL-1 level, down-regulated IL-4 level in burn patients at post-dressing changes, and lowered the protein and mRNA expression of P2X7 receptor in PBMCs, suggesting that puerarin inhibits inflammation and procedural pain induced by dressing changes (Zhang *et al*., 2012a). Puerarin augmented the threshold of thermal and mechanical hypersensitivity, whereas it reduced the stain values of P2X3 protein and mRNA in dorsal root ganglion neurons in the chronic constriction injury model rats, indicating that puerarin reduces chronic neuropathic pain mediated by P2X3 receptors (Xu *et al*., 2012). In addition to P2X3 receptors, P2X2/3 receptors are also involved in the modulation of nociceptive transmission. Nociceptive neurons express the same amount of P2X3 and P2X2/3 receptors. Moreover, P2X3 and P2X2/3 receptors can be expressed separately or together in individual neuron. Puerarin could alleviate the nociceptive transmission mediated by P2X3 and/or P2X2/3 receptors in primary afferent neurons (Liang *et al*., 2010).

Intraperitoneal injection of puerarin (25–200 μM) lowered body temperature and the levels of IL-1β, TNF-α, IL-6, prostaglandin $E_{2,}$ and NO, and blocked the activation of NF-κB and phosphorylation of MAPKs raised by LPS (100 μg/kg) in rats, suggesting that this antipyretic effect was related to the regulation of the synthesis and release of endogenous pyrogen via inhibition of NF-κB activation and the MAPK pathway (Yao *et al*., 2012).

Inhibition of alcohol-induced disorders

The cell viability, cellular lipid accumulation, and the expression of microtubule-associated protein 1 light chain 3 in ethanol-incubated hepatocytes were restored after puerarin treatment. Furthermore, puerarin stimulated AMPK phosphorylation, subsequently blocked S6 ribosomal protein and 4E-binding protein 1 that were the mammalian targets of rapamycin (mTOR), and recovered autophagy in ethanol-treated cells. These results indicate that the protective mechanism is involved in the AMPK/mTOR pathway (Noh *et al*., 2011). In bone marrow stromal cells treated with alcohol, puerarin inhibited adipocytes number, triglycerides production, and PPARγ mRNA expression, whereas it enhanced alkaline phosphatase activity, osteocalcin content, and osteocalcin mRNA expression, suggesting that puerarin has preventive effects on alcoholism-related disorders.

An *in vivo* study indicates that puerarin acts as a weak benzodiazepine site antagonist (Overstreet *et al*., 2003). Puerarin counteracted anxiety related to alcohol withdrawal in rats, which evidently enhanced the social interaction and locomotor activity deceased by withdrawal from chronic alcohol diet, as did flumazenil (an antagonist of benzodiazepine) and SB 242084 (an antagonist of 5-HT_{2C}) (Rezvani *et al*., 2003). Alcohol induced marrow necrosis, the hypertrophy and proliferation of adipocytes, and thinner and sparse trabeculae, suppressed hematopoiesis, and augmented empty osteocyte lacunae in the subchondral region of the femoral head in mice, whereas these changes were recovered by puerarin. Collectively, puerarin could suppress bone marrow adipogenesis and inhibit alcohol-induced osteonecrosis (Wang *et al*., 2008). Puerarin could also inhibit alcohol consumption in humans, so it might be a useful agent against excessive alcohol intake (Penetar *et al*., 2012).

Pretreatment with puerarin dose-dependently elevated body weight and liver alcohol dehydrogenase activity in chronic alcoholic mice (Zhang et al., 2010a, 2010b). Oral administration of puerarin (90 and 180 mg/kg) reversed the increased levels of AST, ALT, hepatic gamma-glutamyl transpeptidase, and triglyceride induced by chronic alcohol intake in rats; inhibited alcohol-induced neutrophil infiltration in hepatic lobules and fatty liver; and blocked the up-regulated levels of TNF-α, endotoxin, endotoxin receptors activity, hepatic CD68, CD14, LPS-binding protein, and Toll-like receptor 2/4 as well as the down-regulated distribution of intestinal microvilli and protein expression of intestinal zonula occludens-1 stimulated by alcohol. This protective property against chronic alcoholic liver injury in rats was associated with suppression of Kupffer cell activation, endotoxin gut leakage, and endotoxin receptors expression (Peng *et al*., 2013).

Puerarin could exert the protective effect against acute alcohol-induced liver injury, which might be due to inhibition of oxidative stress (Zhao *et al*., 2010). In addition, puerarin restrained the expression of alpha-smooth muscle actin and TGF-β1 in liver tissue of rats with alcoholic liver injury (Wu *et al*., 2008). Pretreatment with puerarin prolonged the duration of diazepam-induced loss of righting reflex in acute alcoholic rats. Alcohol intoxication markedly reduced gama-aminobutyric acid type A receptor ($GABA_AR$) α1 subunit expression and increased $GABA_AR$ α4 subunit expression in hippocampus, which could be reversed by puerarin (Zhang *et al*., 2010a, 2010b). Puerarin reduced alcohol intake in alcohol-preferring rats (Overstreet *et al*., 2002) and attenuated myocardial levels of phosphokinase (CPK), MDA, and AST and serum levels of CPK and AST, whereas it augmented

SOD and Ca^{2+}-Mg^{2+}-ATPase in myocardium of acute and chronic alcoholism rats. In summary, puerarin protected against myocardial injuries in acute and chronic alcoholism rats (Cui, 2011).

Antioxidant activity

Puerarin scavenged both superoxide anion induced by riboflavin-light and hydroxyl radical induced by Fenton reaction, suppressed oxidative erythrocyte hemolysis and the level of MDA caused by H_2O_2 in erythrocytes, and alleviated oxidative modification of LDL elicited by ultraviolet ray and cupric sulfate, suggesting its antiperoxidation effect and the potential use for prevention of atherosclerosis (Zhu *et al*., 2002). Puerarin prevented Cu^{2+}-induced LDL oxidation *in vitro* (Liu *et al*., 2007), enhanced the production of plasma prostacyclin and high density lipoprotein (HDL), inhibited adhesion and aggregation of platelet, and suppressed the formation of thrombus (Yang *et al*., 1990; Huang *et al*., 1992). Furthermore, puerarin decreased the production of LDL cholesterol and total cholesterol, whereas it increased the production of HDL in the hyperlipidemic rabbit model (Lu and Zhu, 1998). Puerarin could prevent oxidative and nitrative injury stimulated by nitrite–glucose–glucose oxidase in rat heart *in vitro*, which might be due to the abilities of puerarin to scavenge free radical, to prevent protein carbonyl formation, and to block protein nitration (Lu *et al*., 2009). Puerarin blocked oxidative stress via increase of Bcl-2 level, followed by prevention from T2DM-induced liver injury in mice (Yang *et al*., 2009).

The antioxidant and hypocholesterolemic properties of puerarin have been demonstrated in HepG2 cells and C57BL/6J mice. Puerarin evidently inhibited the oxidative modification of LDL, markedly enhanced the mRNA and protein expression levels of LDL receptors, and attenuated 3-hydroxy-3-methylglutaryl coenzyme A reductase transcription and translation in both HepG2 cells and mouse livers. Puerarin notably elevated cholesterol 7α-hydroxylase mRNA expression in the mouse livers (Chung *et al*., 2008), whereas it reduced serum cholesterol content (Hsu *et al*., 2003). Pretreatment with puerarin notably inhibited *tert*-butyl hydroperoxide (*t*-BHP)-induced caspase-3 activation and subsequent apoptosis in Hepa1c1c7 and HepG2 cells. Puerarin enhanced HO-1expression, resulting in cytoprotection against *t*-BHP-induced oxidative injury, which was reversed by silencing Nrf2 expression with specific siRNA. In addition, puerarin evoked the translocation of Nrf2 nuclear and the activation of PI3K. These effects of puerarin on HO-1 expression and PI3K activation were abolished by the conjunct administration of ICI 182,780 and pertussis toxin. These findings demonstrate the cytoprotective effect of puerarin against cellular oxidative injury via an ER-dependent Gβ1/PI3K/Akt-Nrf2 and HO-1 pathway (Hwang and Jeong, 2008).

Puerarin dose-dependently abolished lead-induced nephrotoxicity in rats and reversed other changes involved, such as enhanced ROS generation and lipid peroxidation and depleted intracellular GSH level in rat kidney. Additionally, puerarin elevated urinary lead excretion, reduced the lead levels in serum and kidney (Wang *et al*., 2013), suppressed caspase-3 activity, and promoted NO content and the phosphorylation of Akt and eNOS, leading to improvement of the balance between proapoptotic and antiapoptotic Bcl-2 family proteins and a prevention of mitochondria cytochrome c release in kidney of lead-treated rats. These results indicate that puerarin could protect kidney against oxidative damage by down-regulation of ROS production and up-regulation of kidney GSH level and effectively block lead-stimulated apoptosis in kidney via the PI3K/Akt/eNOS signaling pathway (Liu *et al*., 2012a). These effects might be associated with the ability of puerarin to ameliorate mitochondrial function.

Puerarin could inhibit histopathologic changes, renew antioxidant enzymes activities, lower levels of ROS, MDA, and 8-hydroxydeoxyguanosine, and suppress apoptosis owing to inhibition of caspase-3 activity in the liver of lead-treated rats, suggesting that puerarin exerts its protective effect against lead-induced hepatic injury through amelioration of DNA oxidative damage and apoptosis (Liu *et al*., 2012b). Lead treatment markedly increased the levels of serum ALT and AST, hepatic ROS, and MDA and notably decreased the level of GSH and activities of glutamate-cysteine ligase (GCL), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferase (GST), which were effectively reversed by puerarin administration. In addition, puerarin inhibited the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase, cholesterol 7α-hydroxylase, and LDL receptor; down-regulated the levels of serum cholesterol, liver triglycerides, and LDL; and up-regulated serum HDL level in lead-treated rats. Therefore, puerarin ameliorated lead-induced liver damage and hyperlipidemia by down-regulation of ROS production, up-regulation of antioxidant enzymes activities, and modulation of hepatic lipid syntheses and metabolism gene expression (Liu *et al*., 2011).

Behavioral indicators showed that puerarin evidently inhibited lead-induced neurotoxicity in a dose-dependent manner in imprinting control region mice, reduced lead content in brain and blood, elevated acetylcholinesterase, monoamine oxidase, phosphor-Akt and PKA activities, eNOS and nNOS expression, and NO production in brain, and blocked lead-induced up-regulation of the lipid peroxidation level and depletion of total antioxidant capacity in brain of lead-treated mice. These protective effects were attributed to suppression of oxidative stress, restoration of lead-induced changes in enzymes and transmitters, and modulation of the PKA/Akt/NOS signaling pathway (Liu *et al*., 2013). Pretreatment with puerarin inhibited serum enzymatic activity and hepatic MDA formation, increased GSH content and GST activity, and lowered cytochrome P450 (CYP) 2E1 activity and CYP2E1 protein expression in the liver of CCl4-intoxicated mice. Moreover, puerarin exerted antioxidant effects by scavenging of free radicals and inhibition of lipid peroxidation in the mouse liver. Altogether, puerarin elicited its protective action upon CCl_4-induced liver injury due to inhibition of CYP-mediated CCl_4 bioactivation and antioxidant activity (Hwang *et al*., 2007).

Puerarin inhibited AGE-induced oxidative stress and subsequent apoptosis in human gingival fibroblasts, which might be the mechanism underlying the protective effect of puerarin on periodontal tissues (Xu *et al*., 2011). In PBMCs of asthma patients, puerarin suppressed the activation of the TNF-α and NF-kB pathway, which has a great effect on the pathogenic mechanism of asthma.

Thus, antioxidant puerarin might be useful for asthma patients (Liu *et al*., 2010).

Phytoestrogenic activity

Puerarin is commonly regarded as phytoestrogen because of its estrogenic activity in certain animal models (Hsu *et al*., 2009). A short-term injection of puerarin (0.7 mg/kg) up-regulated the number of uterine glands in ovx immature rats, and a long-term injection of puerarin (7 mg/kg) obviously increased the percentage of cornified cells in mature female rats, indicating that puerarin has an estrogenic activity (Malaivijitnond *et al*., 2010). Oral administration of puerarin for 3 months up-regulated both bladder and urethra pressures, reduced obesity, and augmented uterine weights in ovx rats. All of these changes elicited by puerarin were consistent with but weaker than 17β-estradiol (E_2). These results demonstrate that puerarin could ameliorate urethral closure, which might be beneficial to the treatment of postmenopausal urge incontinence (Thielemann *et al*., 2010). Puerarin markedly elevated weight, IGF-1 and C3 expression, progesterone receptor mRNA levels in uterine, and the level of total antioxidant capacity in serum, whereas it attenuated MDA and lymphocytes DNA damage in ovx rats, suggesting that puerarin has uterotropic effects and might be an effective therapeutic agent against endometrial hyperplasia and premenstrual syndrome (Rachoń *et al*., 2007; Tang *et al*., 2012).

Puerarin blocked estrogen-response element (ERE)-reporter transcriptional activity in human MG-63 osteoblastic cells overproducing estrogen receptor β (ERβ), suggesting an antagonistic effect on ERE reporter transcription via ERβ (Tang *et al*., 2008). Additionally, puerarin attenuated the levels of insulin resistance and the androgen synthesis induced by dexamethasone in ovarian theca cells of porcine follicles, so it might be useful for the treatment of polycystic syndrome (Gao *et al*., 2009b).

The overexpression of Aromatase P450 (P450arom) is closely relevant to the development of endometriosis. Puerarin blocked the expression of P450arom and decreased the estrogen level in ectopic endometrium, resulting in suppression of the growth and development of endometriosis in rats (Chen *et al*., 2011b). Puerarin inhibited the mRNA and protein expression of P450arom in Ishikawa and RL95-2 cell lines, suppressed the mRNA expression of c-jun, a critical substance for P450arom overexpression, and down-regulated AP-1 (c-jun dimer) (Li *et al*., 2008b), suggesting that puerarin might be a potential medication for the treatment of endometriosis and the mechanism involved might be due to the down-regulation of P450arom expression related to inhibition of transcription factor AP-1 or c-jun (Yu *et al*., 2008).

E_2 (10^{-8} mol/L) up-regulated the accumulation of MMP-9, down-regulated tissue inhibitor of metalloproteinase 1 accumulation, and subsequently enhanced the invasiveness of endometriotic stromal cells (ESCs), all of which were evidently reversed by administration of puerarin (10^{-9} mol/L). Puerarin also suppressed E_2-induced endometriotic tissue angiopoiesis and the accumulation of ICAM-1 and vascular endothelial growth factor. These results indicate that puerarin could interfere with tissue invasion of ESCs and angiogenesis of ectopic endometrial tissues induced by E_2 (Wang *et al*., 2011a). Moreover, puerarin was able to bind to ERs, while the binding ability was almost one third of E_2. Puerarin retarded human ESCs proliferation evoked by E_2-BSA possibly through prevention of the expeditious, non-genomic, and membrane-mediated ERK pathway and reduction of E_2-BSA-induced expression of certain gene, including Cyclin D1, Cox-2, and Cyp19 (Cheng *et al*., 2012). In addition, puerarin inhibited cell apoptosis, tumor-related gene expression, and angiopoiesis in endometriotic tissue of patients with endometriosis (Yu *et al*., 2009). Therefore, puerarin might be an ideal therapeutic agent for endometriosis.

Anticancer activity and induction of apoptosis

Oral administration of puerarin (0.4 and 0.8 g/kg) down-regulated the levels of serum ALT and AST as well as Bcl-2 mRNA expression, whereas it stimulated apoptosis of activated hepatic stellate cells in rats with liver fibrosis induced by alcohol plus carbon tetrachloride, indicating that puerarin could reverse chemical-induced liver fibrosis by restoration of hepatic injury and induction of cell apoptosis (Zhang *et al*., 2006). In cigarette smoke-exposed rats, puerarin treatment inhibited the level of intra-acinar pulmonary arteries (CMA/IAPA) and alpha-smooth muscle actin expression, increased artery smooth muscle cell (PASMC) apoptosis, and attenuated the mRNA and protein expression of PKC-α. Puerarin ameliorated the cigarette smoke-stimulated pulmonary vascular remodeling probably via activation of the PKC signaling pathway and induction of PASMC apoptosis (Zhu *et al*., 2008b). Puerarin impeded the proliferation of four acute myeloid leukemia cell lines, U937, Kasumi-1, HL-60, and NB4 cells, interfered with cell cycle process (Shao *et al*., 2010), and elevated the apoptosis rate of acute promyelocytic leukemia cell line NB4. The mechanism might be associated with the JNK signal pathway (Tang *et al*., 2010). Puerarin (20 μmol/L) retarded the motility migration, adhesion, and invasion of human oophoroma cells HO-8910, providing a mechanism of puerarin antagonizing the estrogenic insult on the malignant behavior of tumor cells (Han *et al*., 2009).

Abolishing multi-drug resistance (MDR) plays an important role in reversing cancer drug resistance. The expression of MDR1 and the activity of MDR1 promoter were obviously suppressed by puerarin in breast cancer MCF-7/adriamycin (MCF-7/adr) cells, resulting in inhibition of MDR phenotype. Meanwhile, puerarin blocked NF-κB activity and inhibitor-κB degradation. Puerarin induced the transcriptional activities of acetyl-CoA carboxylase, AMPK, and glycogen synthase kinase-3β, whereas it suppressed the phosphorylation of cAMP-responsive element-binding protein and cAMP-responsive element (CRE). Interestingly, compound C (an inhibitor of AMPK) decreased the suppressive effect of puerarin on MDR1 expression. Protein kinase A/H89 (an inhibitor of CRE) blocked the regulation of puerarin on MDR1 protein expression and CRE phosphorylation. These results demonstrate that the NF-κB pathway and CRE transcription-dependent elevation of AMPK correlated with the inhibition of puerarin on MDR1 expression in MCF-7/adr cells (Hien *et al*., 2010). The molecular mechanism for puerarin inhibition of MDR of K562 and K562/AO2 might be related

to suppression of NF-κB activity, survivin, and p-gp expression (Chen *et al*., 2008).

Puerarin blocked the proliferation of HS578T, MDA-MB-231, and MCF-7 breast cancer cells at a 50% cell growth inhibition (GI50) concentration of 46, 71, and 69 μM, respectively. Puerarin was also able to prevent three types of breast cancer cells into the G_0/G_1 phase of the cell cycle and stimulated apoptosis in these cells. The mechanism might be associated with prevention of cell cycle progression and stimulation of apoptosis via the caspase-3 pathway (Lin *et al*., 2009). Puerarin is an ideal candidate as an antitumor agent owing to its induction of apoptosis in various types of human cancer cells (Park and Surh, 2004; Sarkar and Li, 2003). Administration of puerarin dose-dependently and time-dependently inhibited colon cancer HT-29 cellular growth, suppressed c-myc and Bcl-2 expression, enhanced Bax expression and caspase-3 activation, and subsequently induced apoptosis in HT-29 cells (Yu and Li, 2006).

Other activities

Clinically, puerarin could be used in the management of hypoxia-induced pulmonary hypertension, which was associated with the mitochondria-dependent pathway. Mitochondria-dependent apoptosis was inhibited by hypoxia. In contrast, after puerarin (50 mM or more) treatment, apoptosis was observed in hypoxic PASMCs, which was accompanied by depolarized HPASMC mitochondria, PT promotion, cytochrome c release from the mitochondria, caspase-9 activation, and Bcl-2 down-regulation with concurrent Bax up-regulation (Chen *et al*., 2012a). In accordance with those results, puerarin induced apoptosis in rat PASMCs via the mitochondrial pathway (Zhang *et al*., 2011e). Puerarin impeded proliferation and promoted hypoxia-evoked apoptosis in rat PASMCs, which might be correlated with enhancement of caspase-3 activity and up-regulation of the protein and mRNA expression of Kv 1.5 (Chen *et al*., 2011a).

In cultured B16 melanocytes, puerarin notably lowered melanin content, tyrosinase activity, and the expression of microphthalmia-associated transcription factor (MITF) and its target genes. Puerarin also blocked MITF and tyrosinase protein expression and inhibited mushroom tyrosinase activity by 88% at 4.8 mM. Puerarin might elicit hypopigmentation through suppression of tyrosinase activity directly and melanogenesis-related gene expression and might be useful for the development of functional cosmetics related to antimelanogenesis (Choi *et al*., 2010).

The antiarrhythmic effect of puerarin has been investigated *in vitro* and *in vivo*. Puerarin dose-dependently inhibited inward rectifier potassium channel (I_{K1}) currents in Xenopus oocytes. Consentaneously, puerarin (1.2 mM) effectively blocked I_{K1} inward currents in rat ventricular myocytes. Puerarin competed with barium (an open-channel inhibitor of I_{K1}) to suppress I_{K1} currents. Moreover, puerarin antagonized ouabain-induced ventricular extrasystole/tachycardia in guinea-pigs and chloroform-epinephrine-induced cardiac arrhythmias in rabbits (Zhang *et al*., 2011b).

Puerarin (4, 8, and 16 μM) dose-dependently inhibited H_2O_2 (700 μM)-induced apoptosis in nerve growth factor differentiated PC12 cells, whereas it elevated phospho-BAD and phospho-Akt production, which could be abolished by wortmannin, indicating that puerarin might elicit its neuroprotective effect via the PI3K/Akt signaling pathway (Zhang *et al*., 2012c). The increased intracellular calcium concentration $[Ca^{2+}]i$, phosphorylation of neurofilament (NF), and down-regulated rates of axonal transport of NFs induced by glutamate (Glu) in rat primary hippocampal neurons could be reversed by puerarin, which also significantly inhibited Glu-induced activation of Cdk5. These results suggest the neuroprotective effect of puerarin against Glu-evoked NF axonal transport impairment by inhibition of the augmented $[Ca^{2+}]i$ and Cdk5 activation (Zhou *et al*., 2010). Puerarin had an antagonistic action on LPS-activated N9 microglia cells and might be beneficial to the treatment of neurodegenerative disorders (Bai *et al*., 2010). Puerarin evidently enhanced cultured Schwann cell viability, increased the density of myelinated axons, action potential area, and nerve conductive velocity, and consequently promoted nerve growth, which might be a potential medication for recovery of regenerating peripheral nerves (Hsiang *et al*., 2011).

Puerarin delayed the onset of endothelial progenitor cells (EPCs) senescence *in vitro*, enhanced EPCs proliferation and colony expansion capacity, and augmented the activity of telomerase and Akt phosphorylation. Intriguingly, puerarin-mediated telomerase activity was evidently prevented after pretreatment with wortmannin/LY294002 (PI3K blockers). Consequently, the mechanism of puerarin suppressing the onset of EPCs senescence might involve the activation of telomerase via the PI3K/Akt pathway, and the preventive effect of puerarin from EPCs senescence *in vitro* might be useful for potential cell therapy (Zhu *et al*., 2008a).

Puerarin significantly decreased serum ALT, AST, and total bilirubin activities as well as extracellular matrix production; up-regulated albumin and total protein content in CCl4-induced HF rats; and alleviated CCl_4-induced liver injury from the results of pathological examination. Moreover, puerarin markedly lowered the levels of collagen type I, type III precollagen (PCIII), and hydroxyproline as well as the expression of tissue inhibitor of metalloproteinase 1, p-PI3K, and p-Akt in the liver of HF rats, whereas it elevated the expression of PPAR-γ and MMP-2. The protective effect of puerarin against CCl_4-induced hepatotoxicity in HF rats is due to the ability of suppressing collagen accumulation by inhibition of the PI3K/Akt pathway and modulation of PPAR-γ expression (Guo *et al*., 2013).

Puerarin obviously attenuated the content of total cholesterol and triglyceride in liver, serum level of leptin, inflammatory reaction in liver, and hepatic steatosis (Zheng *et al*., 2008), whereas it elevated the mRNA expression of leptin receptor and the level of phosphorylated Janus kinase 2 (P-JAK2)/phosphorylated signal transducers and activators of transcription 3 (P-STAT3) expression in the liver of non-alcoholic fatty disease rats. These results indicate that puerarin plays an antagonist effect against non-alcoholic fatty liver injury via leptin and JAK2/STAT3 pathway (Zheng *et al*., 2009).

Pretreatment with puerarin lessened the up-regulated ceramide accumulation and subsequently lowered the elevated levels of p-p38 protein and free calciumion, leading to reduction of augmented DNA damage in HaCaT cells stimulated by UVB (Huang *et al*., 2012b). Irradiation with ^{60}Co-gamma ray induced injury of sertoli cells and spermatogenic cells, notably elevated

the serum level of follicle-stimulating hormone, and significantly diminished the serum level of inhibin beta and the mRNA expression of inhibin beta in testicular tissue in mice. Interestingly, these alterations were reversed by puerarin (230 and 450 mg/kg) administration, indicating the protective effect of puerarin on ^{60}Co-gamma-evoked injury of Stetoli cells in mice (Wang *et al*., 2012c). Furthermore, puerarin could prevent from secondary spinal cord injury, which accelerated Bcl-2 expression and SOD activity, whereas it suppressed Bax expression and MDA level in rat models of spinal cord injury (Heng *et al*., 2009).

Puerarin dose-dependently enhanced the proliferation activity and number of vascular endothelial cells and elevated the levels of proliferation index and proliferating cell nuclear antigen expression (Zhang *et al*., 2008c). In TNF-α-stimulated human umbilical vein endothelial cells (HUVECs), puerarin suppressed the protein and mRNA expression of vascular cell adhesion molecule-1, ICAM-1, and endothelial leukocyte adhesion molecule 1 (E-selectin), which might be relevant to the prevented transcriptional level of NF-kB activation (Hu *et al*., 2010). Puerarin (1–100 μM) reversed the elevated activities of calpain and caspase-3, the declined HO activity, and HO-1 mRNA expression induced by HG (33 mm) in HUVECs. Intriguingly, protoporphyrin IX zinc (II) (an inhibitor of HO-1) blocked the preventive effect of puerarin, suggesting that puerarin inhibited HG-induced endothelial cell apoptosis owing to the modulation of HO-1 expression (Chen *et al*., 2012d).

CONCLUSION

Puerarin, the major bioactive ingredient of Gegen, is available in common foods and is used as alternative medicine. It has been demonstrated to possess a wide spectrum of pharmacological effects such as vasodilation, cardioprotection, neuroprotection, antioxidant, anticancer, antiinflammation, alleviation of pain, promotion of bone formation, inhibition of alcohol intake, and attenuation of insulin resistance. It is suggested that puerarin may be a valuable therapy option for the prophylaxis and treatment of various diseases, including arrhythmia, diabetes and diabetic complications, PD, AD, osteonecrosis, hyperlipidemia, endometriosis, and cancer.

However, further studies are required for puerarin development. Molecular mechanisms and targets underlying certain pharmacological properties of puerarin are still unknown. The poor solubility, low oral bioavailability, and short elimination half-life of puerarin limit its clinical application further. In addition, there have been side effects reported on puerarin. For example, puerarin (5 and 10 μM) affected mouse embryonic development and viability (Chen and Chan, 2009). Puerarin injection induced hemolysis clinically (Yue *et al*., 2008).

The documents summarized earlier potently support the view that puerarin has a broad application prospect. Nevertheless, further researches are needed to explore the direct molecular mechanisms and targets, improve oral bioavailability, and attenuate side effects.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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