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•Research article•

The cardiovascular protective effect and mechanism of calycosin and its derivatives

PAN Li[1], ZHANG Xuan-Fen[2*], WEI Wan-Sheng[1], ZHANG Jing[1], LI Zhen-Zhen[1]

[1] *Department of Cardiopulmonary Bypass, Lanzhou University Second Hospital, Lanzhou 730000, China;*
[2] *Department of Orthopaedic Surgery, Lanzhou University Second Hospital, Lanzhou 730000, China*

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[ABSTRACT] Cardiovascular disease is the main cause of mortality and morbidity in the world, especially in developing countries. Drug therapy is one of the main ways to treat cardiovascular diseases. Among them, great progress has been made in the treatment of cardiovascular diseases with traditional Chinese medicine. In terms of experimental research, the mechanism of traditional Chinese medicine in the treatment of cardiovascular diseases has been thoroughly discussed *in vitro* and *in vivo*. In terms of clinical treatment, traditional Chinese medicine with flavonoids, saponins and alkaloids as the main effective components has a definite effect on the treatment of cardiovascular diseases such as arrhythmia, myocardial ischemia, angina pectoris and myocardial infarction, with high safety and good application prospects. With the further research on the effective ingredients, mechanism and adverse reactions of traditional Chinese medicine, it will be beneficial to the effectiveness of traditional Chinese medicine, reduce side effects and promote the modernization of traditional Chinese medicine. Calycosin and its derivatives, the main bioactive flavonoids in *Astragalus membranaceus* have multiple biological effects, such as antioxidant, pro-angiogenesis, anti-tumour, and anti-inflammatory effects. Based on the above biological effects, calycosin has been shown to have good potential for cardiovascular protection. The potent antioxidant effect of calycosin may play an important role in the cardiovascular protective potential. For injured cardiac myocytes, calycosin and its derivatives can alleviate the cell damage mainly marked by the release of myocardial enzymes and reduce the death level of cardiac myocytes mainly characterized by apoptosis through various mechanisms. For vascular endothelial cells, calycosin also has multiple effects and multiple mechanisms, such as promoting vascular endothelial cell proliferation, exerting vasodilating effect and directly affecting the synthesis function of endothelial cells. The present review will address the bioactivity of calycosin in cardiovascular diseases such as protective effects on cardiac myocytes and vascular endothelial cells and elucidate main mechanism of calycosin and its derivatives to exert the above biological effects.

[KEY WORDS] Cardiovascular disease; Calycosin; Cadiac myocytes; Vascular endothelial cells; Protective effect
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Introduction

Cardiovascular disease (CVD) is one of the leading cause of death worldwide, especially in developing countries, where the prevalence has increased significantly [1]. The global number of deaths from CVD has increased during the past decade by 12.5% [2]. Between 1990 and 2020, the burden of CVD in the developing countries is growing [3]. These changes are driven by population growth and aging populations, with the largest number occurring in countries of South and East Asia because of their large and growing populations [4]. Elevated blood lipid levels, hypertension, elevated blood glucose levels, overweight, unhealthy dietary habits, cigarette smoking, and insufficient regular exercise are established primary causal factors of CVD [5]. Over 95% of all CVD deaths are attributable to 6 conditions: ischemic heart disease (IHD), stroke, hypertensive heart disease (which ultimately results in heart failure), cardiomyopathy, rheumatic heart disease (RHD), and atrial fibrillation (AF) [4, 6, 7]. The treatment methods of CVD mentioned above generally include drug therapy, interventional methods and surgical techniques that improve the health of patients from different perspectives and mechanisms [8]. Among them, drug therapy is one of the main ways to treat CVD. In recent years, with the wide application of high and new technologies and biotechnology in drug research, natural medicines show great potential in the application and discovery of cardiovascular drugs [9].

Astragalus membranaceus has been widely studied and

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[*Corresponding author] E-mail: zhxf9304@126.com
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has been shown to have important pharmacological effects, such as protecting injured myocardium, inhibiting ventricular remodelling and regulating blood pressure [10]. However, the components of *Astragalus membranaceus* are complex and varied and more than 200 compounds have been identified from *Astragalus membranaceus* [11]. The major active extracts include flavonoids, triterpene saponins and polysaccharides [11, 12]. *Astragalus membranaceus* are a rich source of flavonoids. Studies have shown that flavonoids from Astragalus membranaceus have multiple physiological effects, such as antioxidant, antitumor, anti-inflammatory, anti-osteoporotic effects, and so on [13]. Especially, flavonoids that serve as potent antioxidants can inhibit the production of reactive oxygen species (ROS) and free radicals that are responsible for many human diseases [14].

Currently, exploring the pharmacological effects of a single bioactive component for the treatment of cardiovascular diseases and validating the specific therapeutic target are hot research topics. Calycosin and its derivatives are the main bioactive flavonoids in *Astragalus membranaceus* [15]. Studies have confirmed that calycosin has multiple biological effects, such as antioxidant [16], pro-angiogenesis [17], anti-tumour [18], and anti-inflammatory [19, 20] effects. Based on the above biological effects, current research has been increasingly focused on the protective effects of calycosin on cells and organs. For example, a recent study showed that calycosin has a protective effect against high-fat diet-induced liver damage [21]. In the nervous system, calycosin can activate the transient receptor potential canonical 6 (TRPC6) pathway and other protective proteins to alleviate cerebral ischaemia-reperfusion injury and other injuries [22]. Furthermore, the protective effect of calycosin on the cardiovascular system has also received attention. It has been shown, preliminarily, that calycosin can antagonize hypoxic injury in myocardial ischaemia and increase the myocardial survival rate. However, existing studies have only focused on the preliminary role of calycosin in ischaemic diseases, myocardial hypertrophy and viral myocarditis, while comprehensive and in-depth experimental studies have not been carried out to elucidate its molecular mechanism and determine its effects on other types of myocardial damage.

In recent years, studies on the effects of calycosin on the cardiovascular system have been carried out, and calycosin has been shown to have good potential for cardiovascular protection. Therefore, this review focuses on the bioactivity of calycosin in cardiovascular diseases and reviews previous studies of calycosin to provide a theoretical basis for further research and clinical application.

The cardiovascular protective effects of calycosin and its derivatives

The antioxidant effects of calycosin and its derivatives

Oxidative stress is an unbalanced state of ROS production and abnormal regulation of the endogenous antioxidant mechanism [23]. ROS production is affected by multiple factors, including dysfunction of oxidases, such as xanthinoxidase and nicotinamide adenine dinucleotide phosphate (NADPH), transport dysregulation in mitochondria, microsomes and/or the nucleus, neutrophil activation, arachidonic acid metabolism, and auto-oxidation of catecholamines, flavonoids, quinones, and proteins, *etc*. [24]. Mitochondria are considered the main source of ROS production in high-metabolic organs such as the heart. Mitochondrial calcium activates the dehydrogenases in the Krebs cycle involved in oxidative phosphorylation and maintains a low level of nicotinamide adenine dinucleotide (NADH) accumulation, thus contributing to cellular energy balance and maintaining adequate cardiac function [25]. In cardiac diseases, especially in heart failure, this process is disrupted, and mitochondrial Ca^{2+} uptake disorders eventually lead to NADPH oxidation and ROS production and accumulation [26, 27]. In addition, ROS can also be produced through the metabolism of drugs and exogenous substances, such as anticancer drugs, among which doxorubicin, which is related to the development of cardiomyopathy, is the most representative [28]. Oxidative stress is an important common mechanism of myocardial injury caused by various aetiologies, such as hypertension, hyperglycaemia, ischaemia-reperfusion injury, and chemotherapy drugs, and is closely related to the subsequent occurrence and development of cardiac failure. Abnormally elevated ROS can cause many negative effects on cardiac function [29]. In myocardial tissues, ROS directly damages the electrophysiology and contractile structure of myocardial cells by modifying the core proteins of the excitation-contraction coupling mechanism, including L-type calcium channels, sodium channels, potassium channels and sodium/calcium exchangers, and causes an energy deficiency in cardiac myocytes by affecting the function of proteins involved in energy metabolism [23]. ROS leads to the activation of multiple signalling pathways related to cell death, cardiac fibroblast proliferation, matrix metalloproteinase activation, mitochondrial deoxyribonucleic acid (DNA) damage, mitochondrial dysfunction, impaired calcium regulation and cardiac hypertrophy [30-33]. These effects eventually lead to abnormal adaptive myocardial remodelling and cardiac dysfunction [34]. In addition, recent studies have proposed that ROS can directly modify micro ribonucleic acid (microRNA), leading to changes in protein expression levels and the disruption of downstream gene regulation in corresponding tissues, thus leading to proteome remodelling and metabolic changes in cardiac tissues [35-37]. In addition to abnormally regulating related biomolecules and signaling pathways, ROS can also trigger cascade reactions of oxidative stress and expand the oxidative stress injury mentioned above, through producing other types of bioactive molecules (aldehydes), ROS induced ROS release and ROS mediated paracrine signals [36, 38].

The abnormal increase in ROS and clearance barriers are the main factors of oxidative stress injury, while flavonoids have been shown to be the main effective components in *Astragalus membranaceus* responsible for antioxidation and

free radical elimination [12]. Recently, studies have adopted electron spin resonance, chemiluminescence and other methods to test the bioactivity of anti-superoxide anion radicals in a few major types of flavonoids. Studies have shown that the antioxidant activity of different flavonoids by the order of strength is quercetin > kaempferol > isorhamnetin > isoquercitrin > rutin > calycosin > vitamin C > (3R)-8, 2′-dihydroxy 7, 4′-dimethoxyisoflavanone > formononetin > ononin > 2′, 4′-dimethoxy-3′-hydroxyl-isoflavanone-6-O-*β*-glucoside > (6aR, 11aR)-10-hydroxyl-3, 9-dimethylnissolin > (6aR, 11aR) 9, 10-dimethylnissolin-3-O-*β*-D-glycoside [39]. These results suggest that calycosin has relatively strong antioxidant activity and is also superior to standard antioxidant vitamin C. Recent studies have more accurately discriminated, from the perspective of quantum chemistry, the antioxidant activity of four major flavonoids, from which it was confirmed that the antioxidant activity of calycosin is stronger than that of three other antioxidants (calycosin > calycosin-7-glucoside > formononetin > ononin) [15]. The antioxidant bioactivity of calycosin is related to its chemical structure. The C-ring $\Delta^{2,3}$-double bond and 4-carbonyl group in calycosin are all bioactive antioxidant groups. They can remove oxidative free radicals, reduce malondialdehyde (MDA) (lipid peroxidation product), protein carbonyl (amino acid oxidation product) and ROS levels, and increase superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) levels. It can also enter the lipid bilayer of biological membranes to ameliorate lipid peroxidation induced by free radicals [40]. Further studies suggest that calycosin may stabilize the membrane structure and enhance membrane integrity, which can reduce the lipid fluidity of the cell membrane and hinder the diffusion of free radicals, thereby reducing oxidative damage in cells [19, 41]. Using biological experiments, it was also shown that calycosin has a good antioxidant effect and that its cellular protective effect in multiple pathological states is mainly antioxidative. Li *et al*. showed that calycosin has a significant inhibitory effect on H_2O_2-induced oxidative stress in human liver parenchymal cells and can significantly alleviate oxidative damage in hepatic cells [42]. Recent studies on the neuronal protective effects of calycosin indicate that calycosin can alleviate oxidative stress and the inflammatory response of neurons in the hippocampus of a mouse model of Alzheimer′s disease by activating the protein kinase C pathway, thereby improving cognitive function [43]. In addition, in myocardial injury caused by ischaemia and hypoxia, calycosin can activate the Akt/PI3K and Nrf2/HO-1 signalling pathways, with myocardial protective effects; these pathways are important in organism oxidation and antioxidative regulation [44]. Therefore, the antioxidative activity of calycosin may play an important role in its cardioprotective potential.

The protective effects of calycosin and its derivatives on cardiac myocytes

Cardiovascular diseases are a large group of pathophysiological states with multiple risk factors and aetiologies, but at the cellular level, various cardiovascular diseases often have a common basis of cell damage, such as oxidative stress in cardiomyocytes or endothelial cells, substrate metabolism and energy consumption, cell survival and apoptosis, and autophagy [45]. Therefore, the treatment of cardiovascular diseases mainly includes two directions: intervention for the aetiologies of cardiovascular diseases and protective measures for the injury effects (organ level, cellular level or molecular level). Based on the multiple biological effects of calycosin and its derivatives, the protective effect of calycosin on cardiovascular cells has been studied.

In vitro effects

For *in vitro* cardiomyocytes affected by different injury factors (H_2O_2, hypoglycaemia, hypoxia, and viruses, *etc*.), calycosin and its derivatives exhibit a common effect, that is, to reduce cell damage, predominantly indicated by the release of myocardial enzymes, and to reduce apoptosis-dominated cardiomyocyte death levels; furthermore, calycosin may play an anti-injury role through multiple mechanisms. For example, in H9C2 cells under hypoxic and hypoglycaemic conditions, pretreatment with calycosin can reduce the released level of the cell damage indicator lactate dehydrogenase (LDH) (Table 1), improve the cell survival rate, and increase the protein expression levels of phosphorylated Akt/PI3K, Nrf-l, Nrf-2, and HO-1, which suggests that the myocardial protective effect of calycosin in this process is related to the direct activation of the PI3K/Akt and Nrf-2/HO-1 pathways [44]. In addition, these pathways are important in cell proliferation and in the balance of oxidation and antioxidation. In another study, the cellular protective effect of calycosin is considered to be related to its phytoestrogen-like effects and the study first demonstrates that calycosin can inhibit the oxidative stress-induced apoptosis of cardiac muscle cells (H9C2 cells) *in vitro* and the protective effect on the heart occur through oestrogen receptor *α* (ER*α*) binding to ER*β* and upregulating its expression, thereby activating the Akt signalling pathway [46]. For infective myocardial injury, calycosin can inhibit coxsackie virus replication in African green monkey kidney heteroploid cell (Vero cells), and its antiviral activity and specificity are all superior to the traditional antiviral drug ribavirin (antiviral activity analysis showed that its half-maximal inhibition (IC_{50}) was 25 μg·mL^{-1} and that the ratio of cytotoxicity to antiviral activity was 5.7), suggesting that it plays a role in cell protection through direct antiviral effects [47].

In vivo effects

In recent years, to further explore the myocardial protective effect and mechanism of calycosin, increasingly more *in vivo* experiments have provided more accurate and reliable evidence from different aspects.

Myocardial ischaemia-reperfusion injury is the main pathological of ischaemic heart disease develops and is associated with the development of myocyte necrosis, arrhythmia, myocardial stunning, endothelial dysfunction, and microvascular complications, *etc* [48-50]. Calycosin has been shown to have a good protective effect on ischaemic and reperfused myocardium: On the one hand, calycosin can improve cardi-

Table 1 *In vitro* myocardial protective effects of calycosin and its derivatives

Chemical components	Cardiac injury inducer	Drug administration method	Cell type	*In vitro* effect	Signalling pathway	References
Calycosin	H_2O_2	5, 10, 20 μmol·L^{-1} pretreatment for 24 hours	H9C2 cells	Cardiomyocyte apoptosis ↓ ERα/β expression levels ↑Akt↑	Akt signalling pathway	[46]
Calycosin -7-*O*-*β*-D-glucopyranoside	Coxsackie virus B3	25 μg·mL^{-1}	African green monkey kidney heteroploid cells (Vero cells)	Virus replication ↓		[47]
Calycosin	Hypoglycaemia and hypoxia	0.01,0.1,1,10 μmol·L^{-1} pretreatment for 2 hours	H9C2 cells	LDH↓ Cell survival rate↑ Phosphorylated Akt, PI3K proteins↑ Nrf2, HO-1 proteins↑	PI3K/Akt signalling pathwayNrf2/HO-1 signalling pathway	[44]

ac function after ischaemia. For example, it increases the ejection fraction (EF), fractional shortening (FS), and left ventricular end systolic pressure (LVESP) and decreases left ventricular end diastolic pressure (LVEDP) (Table 2). Furthermore, it can induce the expression of vascular endothelial growth factor (VEGF) and CD31 and promote the angiogenesis of ischaemic myocardium [51]. On the other hand, oxidative stress is an important mechanism for myocardial ischaemia-reperfusion injury. Calycosin can significantly reduce the level of MDA, the lipid oxidation product of the cell membrane. It also increases the level of the protective antioxidant product SOD, thus inhibiting the release of myocardial enzymes including creatine kinase (CK) and LDH, *etc* [52-54]. The P13K/Akt signalling pathway is considered an important pathway involved in the regulation of cardiomyocyte apoptosis and myocardium tissue protection. In addition, calycosin can increase the protein phosphorylation level of P13K and Akt during ischaemia-reperfusion injury, suggesting that calycosin may exert a myocardial protective effect through activating the P13K/Akt signalling pathway in ischaemia-reperfusion injury [52].

In addition to its protective effect on acute ischaemic injury, calycosin also has a protective effect on some maladaptive changes in myocardial tissue. In a mouse model of myocardial hypertrophy constructed by isoproterenol induction and aortic constriction, calycosin significantly reduced markers of cardiac hypertrophy (atrial natriuretic peptide and myosin heavy chain-*β*) and inhibited myocardial remodelling. Additionally, the activation of the mitogen-activated protein kinase (MAPK) and AKT signalling pathways in mouse myocardium tissue was significantly attenuated, suggesting that calycosin may reduce cardiac hypertrophy induced by isoproterenol or aortic coarctation by inhibiting AKT/GSK3*β* and extracellular signal-regulated kinase (ERK) signalling [55].

For infective myocardial injury, calycosin showed multiple positive effects, such as anti-viral, anti-apoptosis and anti-inflammatory properties. In a mouse model of coxsackie virus B3 infection, calycosin-7-*O*-*β*-D-glucopyranoside (CCGR) treatment increased myocardial contractile force and cardiac EF. In addition, the progression of heart failure in infected mice was inhibited by increasing the systolic ventricular septal thickness, left ventricular posterior wall thickness and left ventricular volume. Overall, the symptoms of viral myocarditis were reduced, and the survival rate was improved (77.7% in the treatment group and 44.4% in the non-treatment group). This study further suggested that CCGR could play a protective role in myocardial tissue in viral myocarditis by significantly reducing viral titres, reducing myocardial cell oedema and inhibiting myocardial cell necrosis and monocyte infiltration caused by viral infection [47].

The protective effects of calycosin and its derivatives on vascular endothelial cells

The influence of calycosin on vascular endothelial cells has multiple effects and multiple mechanisms. First, calycosin, as a phytoestrogen, can bind to ER-positive cells in the vascular endothelium and exert its role as a selective oestrogen receptor modulator (SERM) by increasing ERK1/2 phosphorylation and activating the MAPK signalling pathway, thereby promoting vascular endothelial cell proliferation [57]. Second, calycosin can interfere with ion channels. It has been shown that calycosin has a vasodilating effect, which is not endothelium-dependent and independent of intracellular calcium ion release. Calycosin is a non-competitive calcium channel blocker that primarily blocks voltage-gated calcium ion channels and receptor-gated calcium channels [58]. Tseng [59] *et al*. demonstrated that calycosin could activate the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels of human umbilical vein endothelial cells, thereby increasing the hyperpolarization of endothelial cells and the production of nitric oxide and mediating endothelium-dependent vasodilation. In addition, calycosin also has protective effects on endothelial cell injury induced by various factors. For endothelial cell injury induced by bacterial endotoxin, calycosin can increase the production of nitric oxide, reduce phosphorylated myosin light chain, reduce Rho/Rock signalling pathway activation-induced cytoskeleton remodelling by in-

Table 2 ***In vivo*** **myocardial protective effects of calycosin and its derivatives**

Chemical components	Drug administration methods	Dose	Animal Model	Protective effects	Signalling pathway	References
Calycosin	Intraperitoneal injection	40 mg·kg^{-1}·d^{-1} 1 t·d^{-1}, 3 days	Isoproterenol-induced myocardial infarction mouse model	MPO↓ (Neutrophils seepage reduction) MDA↓ (Lipid peroxidation attenuation)		[53]
Calycosin	Intraperitoneal injection	0.5 mg·kg^{-1} 1 mg·kg^{-1} 2 mg·kg^{-1} 4 mg·kg^{-1} 1 t·d^{-1}, 28 days	myocardial ischaemia rat model	Cardiac function↑ VEGF expression↑		[51]
Calycosin-7-*O*-*β*-d glucoside	Intravenous injection	30 mg·kg^{-1} (high dose) 15 mg·kg^{-1} (low dose) pretreatment once	Myocardial ischaemia-reperfusion rat model	Cardiac function↑ Area of myocardial infarction ↓ CK, LDH, MDA↓ SOD↑ caspase-3, caspase-9↓ phosphorylated PI3K p85/Akt↑	PI3K/Akt signalling pathway	[52]
Calycosin-7-*O*-*β*-D-glucopyranoside	Intragastric gavage	24 mg·kg^{-1} 1 t·d^{-1}, 14 days	Coxsackie virus B3 viral myocarditis mouse model	Survival rate, cardiac function↑ Mononuclear cell infiltration, Virus replication↓		[47]
Calycosin	*Ex vivo* perfusion	0.1 μmol·L^{-1} pretreatment continuous perfusion for 10 min	*Ex vivo* cardiac ischaemia-reperfusion rat model	CK, LDH↓ SOD, SDH↑ MDA↓ Nrf-2/HO-1↓ p-Akt/Akt, p-ERK/ERK↓		[54]
Calycosin	Intragastric gavage	50 mg·kg^{-1} 1 t·d^{-1}, 14 days	Isoproterenol intraperitoneal injection and aorta constriction myocardial hypertrophy mouse model	Cardiac function↑ Marker of myocardial hypertrophy↓ p-Akt/Akt↓ p-GSK3*β*/GSK3*β*↓ p-ERK/ERK↓	Akt/GSK3*β* signalling pathway ERK signalling pathway	[55]
Calycosin	Intraperitoneal injection	1 mg·kg^{-1}·d^{-1} 1 t·d^{-1}, 7 weeks	Abdominal aorta constrictive pressure overload rat model of myocardial hypertrophy	Cardiac function↑ JAK1, STAT3 protein expression↓	JAK/STAT signalling pathway	[56]

hibiting the AKT signalling pathway, and exert protective effects on endothelial cells [60]. Calycosin can also reduce the cytotoxicity and apoptosis of human umbilical vein endothelial cells induced by vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor II (VRI), which is related to the activation of the PI3K/Akt/Bad and BRAF/MEK1/2/ERK1/2 signalling pathways [61]. However, for human umbilical vein endothelial cell apoptosis induced by glycation end products, calycosin can directly increase the expression of anti-apoptotic protein Bcl-2 and simultaneously reduce the expression levels of pro-apoptotic proteins Bax and Bad, thereby inhibiting endothelial cell apoptosis [62, 63].

In addition to these protective effects on endothelial cells, calycosin can also directly affect endothelial cells (Fig.1). Calycosin-7-glucoside has therapeutic effect on angiotensin II (Ang II)-induced renin-angiotensin-aldosterone system (RAAS) disorder in human umbilical vein endothelial cells by down-regulation of angiotensin-converting enzyme (ACE) expression and increased ACE2 expression [64]. Calycosin can inhibit the synthesis of Ang II-induced thromboxane A2 (TXA2) and prostacyclin (PGI2) in endothelial cells and reduce Ang II-induced endothelial cell injury. In addition, calycosin can inhibit the secretion and expression levels of endothelial cell inflammatory factors induced by tumor necrosis factor-*α* (TNF-*α*), such as intercellular adhesion

molecule-1 (ICAM-1) and its receptor lymphocyte function-associated antigen 1 (LFA-1), thus protecting endothelial cells [65, 66].

The relation of calycosin and its derivatives to inflammation

The pathophysiological mechanism of many cardiovascular diseases is closely related to the inflammatory response. Previous studies have shown that the inflammatory response plays an important role in the occurrence and progression of cardiovascular diseases [67]. In recent years, it has been found that calycosin and its derivatives have potential anti-inflammatory effects. Recent studies have revealed that the inhibition of the NF-κB signalling pathway may be the main mechanism of calycosin's anti-inflammatory effects [68, 69]. By inhibiting the expression of NF-κB and MAPK signalling pathway-related proteins, calycosin-7-glucoside can reduce the production of pro-inflammatory factors including Prostaglandin E2 (PGE2), TNF-α, interleukin-1β (IL-1β) and interleukin-6 (IL-6) in endotoxin-induced macrophagocytes and inhibit the mRNA expression levels of inflammatory mediators inducible Nitric Oxide Synthase (iNOS) and cyclooxygenase-2 (COX-2) [70]. However, studies on the anti-inflammatory effects of calycosin mainly focus on the diseases of the haematologic system, immune system and neurological system. There is no definitive research on the effects of calycosin on the inflammatory response in cardiovascular diseases.

The effect on platelet

Ischemic heart disease and thromboembolic disease are common types of cardiovascular diseases. Platelets play a crucial role in the development of these diseases by participating in thromboembolism development [71]. Thus, anti-platelet is often a crucial part of cardiovascular disease therapy [72]. Studies have shown that calycosin and its derivatives are the main active ingredients in many traditional Chinese medicine compounds widely used in cardiovascular diseases [73-76]. For example, calycosin-7-*O*-β-D-glucoside as one of main active ingredients of BuyangHuanwu decoction (BHD) can inhibit adenosine diphosphate (ADP)-induced platelet aggregation *in vitro* [73]. In addition, the core bioactive components of compound xueshuantong capsule (CXC) act on different aspects of the vascular system to promote blood circulation. Among them, calycosin-7-*O*-β-D-glucoside has been proved to mainly affect extrinsic clotting activity and to have negative effects on red blood cell (RBC) aggregation, RBC deformability, intrinsic clotting activity and platelet aggregation [74]. Although the above studies reveal the effect of calycosin on vascular system, the mechanism underlying it functional activity requires further systematic research.

Conclusion

Calycosin is characterized by low toxicity and diverse biological effects [77] and is a monomer compound with a

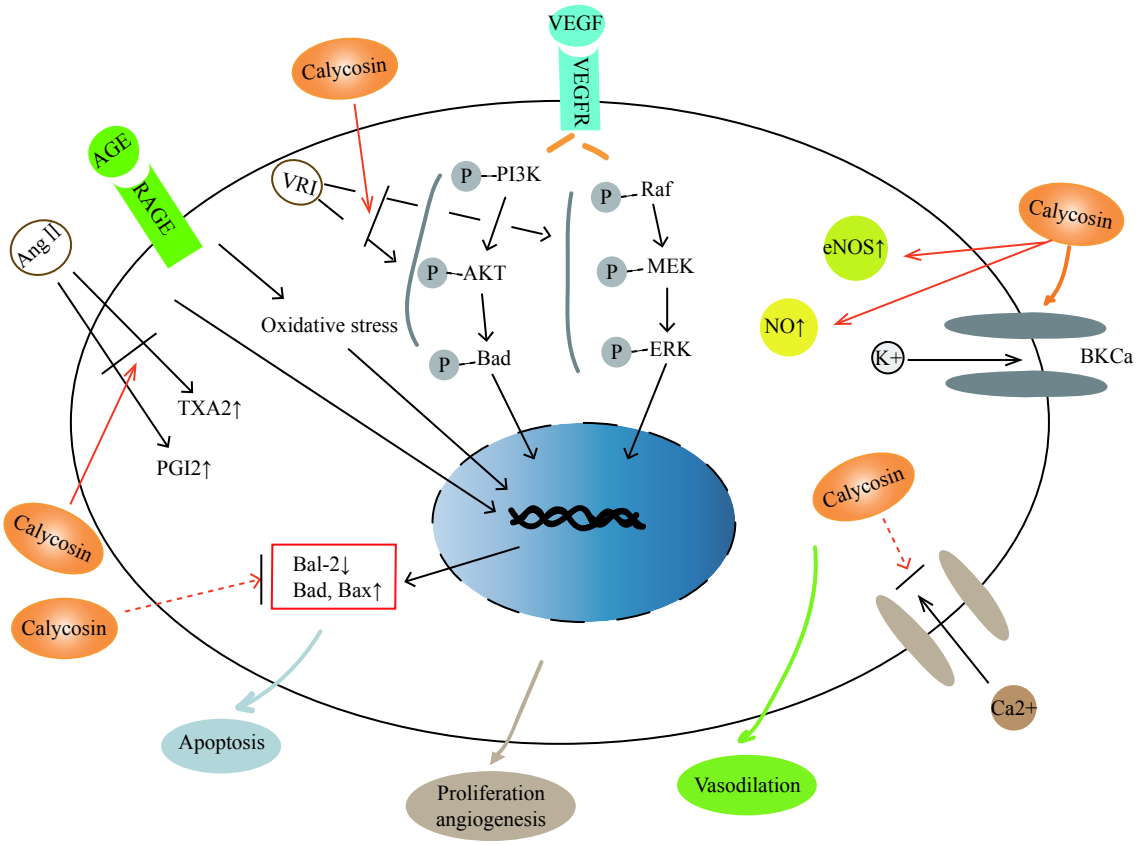

Fig. 1 Schematic chart of the effects and mechanisms of calycosin on vascular endothelial cells

definitive chemical structure, making it more conducive to research and application in pharmacology. Calycosin and its derivatives have been shown to have multiple effects, such as antioxidant, anti-tumour, angiogenesis regulation and anti-inflammation properties. Therefore, calycosin has attracted much attention in many fields, such as tumour treatment [78] and nervous system rehabilitation [22], but there have been few studies on its cardiovascular pharmacological effects. In fact, in recent years, related research has suggested that calycosin and its derivatives have good cardiovascular protective potential. However, existing studies have only focused on the role of calycosin in ischaemic disease, myocardial hypertrophy and viral myocarditis, while the pathological conditions including drug-induced myocardial injury and metabolism-related myocardial injury have not been studied. Second, in the intervention studies of calycosin on various types of myocardial injury, the drug routes and dosages are different; therefore, the pharmacokinetics of calycosin and the correlation between dosage and myocardial protective strength deserve further study. In addition, research on the mechanisms by which calycosin exerts its effects are mostly limited to changes in protein expression level and lack more accurate and in-depth research on signalling pathways and regulatory mechanisms. For example, the main pharmacological effect of calycosin on cardiovascular protection is reducing oxidative stress; however, the specific molecular mechanism of its antioxidative effect is not clear. In addition, calycosin has shown pro-apoptotic effects in tumour studies and anti-apoptotic effects in myocardial protection. Calycosin may have different effects on the corresponding signalling pathways in different pathological conditions. Therefore, the biological effects of calycosin should still be further studied, which could help to further understand its pharmacological characteristics and improve the value of its clinical application.

References

[1] Celermajer DS, Chow CK, Marijon E, *et al*. Cardiovascular disease in the developing world: prevalences, patterns, and the potential of early disease detection [J]. *J Am Coll Cardiol*, 2012, **60**(14): 1207-1216.

[2] Haidong Wang, Mohsen Naghavi, Christine Allen, *et al*. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015 [J]. *Lancet (London, England)*, 2016, **388**(10053): 1459-1544.

[3] Yusuf S, Reddy S, Ounpuu S, *et al*. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization [J]. *Circulation*, 2001, **104**(22): 2746-2753.

[4] Joseph P, Leong D, McKee M, *et al*. Reducing the global burden of cardiovascular disease, part 1: the epidemiology and risk factors [J]. *Circ Res*, 2017, **121**(6): 677-694.

[5] Lloyd-Jones DM, Hong Y, Labarthe D, *et al*. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond [J]. *Circulation*, 2010, **121**(4): 586-613.

[6] Roth GA, Forouzanfar MH, Moran AE, *et al*. Demographic and epidemiologic drivers of global cardiovascular mortality [J]. *N Engl J Med*, 2015, **372**(14): 1333-1341.

[7] Centers for Disease Control and Prevention (CDC). Decline in deaths from heart disease and stroke--United States, 1900-1999 [J]. *MMWR Morb Mortal Wkly Rep*, 1999, **48**(30): 649-656.

[8] Fares MA. Introduction: challenges and advances in cardiovascular disease [J]. *Cleve Clin J Med*, 2017, **84**(12 Suppl 3): 11.

[9] Zhang J. Analysis on research and development of global cardiovascular drugs in the past 6 years [J]. *J China Pharm Univ*, 2018, **49**(06): 760-765.

[10] Wang CH, Chang L, Meng N, *et al*. An analysis of the pharmacological action and clinical application of Huangqi [J]. *Clin J Chin Med*, 2018, **10**(35): 104-107.

[11] Shan H, Zheng X, Li M. The effects of Astragalus membranaceus active extracts on autophagy-related diseases [J]. *Int J Mol Sci*, 2019, **20**(8): 1904.

[12] Li X, Qu L, Dong Y, *et al*. A review of recent research progress on the astragalus genus [J]. *Molecules*, 2014, **19**(11): 18850-18880.

[13] Nijveldt RJ, van Nood E, van Hoorn DE, *et al*. Flavonoids: a review of probable mechanisms of action and potential applications [J]. *Am J Clin Nutr*, 2001, **74**(4): 418-425.

[14] Ahmed M, Eun JB. Flavonoids in fruits and vegetables after thermal and nonthermal processing: A review [J]. *Crit Rev Food Sci Nutr*, 2018, **58**(18): 3159-3188.

[15] Liu JL, Yu HD, Liang YN. Density functional theory investigation on antioxidant activity of flavonoids from astragalus [J]. *Chem & Bioeng*, 2019, **36**(01): 36-40.

[16] Yu L, Wang ZB, Wang QH, *et al*. Research progress on pharmacological effects of flavonoids in astragali radix [J]. *Inf Tradit Chin Med*, 2018, **35**(02): 104-108.

[17] Hu G, Siu SO, Li S, *et al*. Metabolism of calycosin, an isoflavone from astragali radix, in zebrafish larvae [J]. *Xenobiotica*, 2012, **42**(3): 294-303.

[18] Tian J, Duan YX, Bei CY, *et al*. Calycosin induces apoptosis by upregulation of RASD1 in human breast cancer cells MCF-7 [J]. *Horm Metab Res*, 2013, **45**(8): 593-598.

[19] Gao J, Liu ZJ, Chen T, *et al*. Pharmaceutical properties of calycosin, the major bioactive isoflavonoid in the dry root extract of radix astragali [J]. *Pharm Biol*, 2014, **52**(9): 1217-1222.

[20] Cheng CC, Chen YH, Chang WL, *et al*. Phytoestrogen bavachin mediates anti-inflammation targeting Ikappa B kinase-I kappaB alpha-NF-kappaB signaling pathway in chondrocytes *in vitro* [J]. *Eur J Pharmacol*, 2010, **636**(1-3): 181-188.

[21] Duan X, Meng Q, Wang C, *et al*. Effects of calycosin against high-fat diet-induced nonalcoholic fatty liver disease in mice [J]. *J Gastroenterol Hepatol*, 2018, **33**(2): 533-542.

[22] Guo C, Ma Y, Ma S, *et al*. The role of TRPC6 in the neuroprotection of calycosin against cerebral ischemic injury [J]. *Sci Rep*, 2017, **7**(1): 3039.

[23] van der Pol A, van Gilst WH, Voors AA, *et al*. Treating oxidative stress in heart failure: past, present and future [J]. *Eur J Heart Fail*, 2019, **21**(4): 425-435.

[24] Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review) [J]. *Vascul pharmacol*, 2015, **71**: 40-56.

[25] Bertero E, Maack C. Calcium signaling and reactive oxygen species in mitochondria [J]. *Circ Res*, 2018, **122**(10): 1460-1478.

[26] Glancy B, Willis WT, Chess DJ, *et al*. Effect of calcium on the oxidative phosphorylation cascade in skeletal muscle mitochondria [J]. *Biochemistry*, 2013, **52**(16): 2793-2809.

[27] Nickel AG, von Hardenberg A, Hohl M, *et al*. Reversal of mi-

tochondrial transhydrogenase causes oxidative stress in heart failure [J]. *Cell Metab*, 2015, **22**(3): 472-484.
[28] Songbo M, Lang H, Xinyong C, *et al*. Oxidative stress injury in doxorubicin-induced cardiotoxicity [J]. *Toxicol Lett*, 2019, **307**: 41-48.
[29] Munzel T, Camici GG, Maack C, *et al*. Impact of oxidative stress on the heart and vasculature: part 2 of a 3-part series [J]. *J Am Coll Cardiol*, 2017, **70**(2): 212-229.
[30] Gu X, Fang T, Kang P, *et al*. Effect of ALDH2 on high glucose-induced cardiac fibroblast oxidative stress, apoptosis, and fibrosis [J]. *Oxid Med Cell Longev*, 2017, **2017**: 9257967.
[31] Rizzi E, Ceron CS, Guimaraes DA, *et al*. Temporal changes in cardiac matrix metalloproteinase activity, oxidative stress, and TGF-beta in renovascular hypertension-induced cardiac hypertrophy [J]. *Exp Mol Pathol*, 2013, **94**(1): 1-9.
[32] Yu EP, Bennett MR. Mitochondrial DNA damage and atherosclerosis [J]. *Trends Endocrinol Metab*, 2014, **25**(9): 481-487.
[33] Faria A, Persaud SJ. Cardiac oxidative stress in diabetes: Mechanisms and therapeutic potential [J]. *Pharmacol Ther*, 2017, **172**: 50-62.
[34] Ayoub KF, Pothineni NVK, Rutland J, *et al*. Immunity, inflammation, and oxidative stress in heart failure: emerging molecular targets [J]. *Cardiovasc Drugs Ther*, 2017, **31**(5-6): 593-608.
[35] Wang JX, Gao J, Ding SL, *et al*. Oxidative modification of miR-184 enables it to target bcl-xL and bcl-w [J]. *Mol Cell*, 2015, **59**(1): 50-61.
[36] Pinti MV, Hathaway QA, Hollander JM. Role of microRNA in metabolic shift during heart failure [J]. *Am J Physiol Heart Circ Physiol*, 2017, **312**(1): H33-45.
[37] Magenta A, Greco S, Gaetano C, *et al*. Oxidative stress and microRNAs in vascular diseases [J]. *Int J Mol Sci*, 2013, **14**(9): 17319-17346.
[38] Lim HY, Wang W, Chen J, *et al*. ROS regulate cardiac function *via* a distinct paracrine mechanism [J]. *Cell Rep*, 2014, **7**(1): 35-44.
[39] Bian YY, Li P. Study on scavenging activities for superoxide anion radicals and structure-activity relationship of flavonoids from *Astragalus* membranaceus (Fish.) Bge. var. mongholicus (Bge.) Hsiao [J]. *Chin Pharmacol J*, 2008, **4**: 256-259.
[40] Wen XD, Li P, Qian ZM, *et al*. Interaction between three antioxygenic micromolecules and bovine serum albumin [J]. *Acta Chimica Sinica*, 2007, **5**: 421-429.
[41] Guo Q, Rimbach G, Moini H, *et al*. ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids [J]. *Toxicology*, 2002, **179**(1-2): 171-180.
[42] Li J, Han L, Ma YF, *et al*. Inhibiting effects of three components of *Astragalus* membranaceus on oxidative stress in Chang Liver cells [J]. *Chin J Chin Mater Med*, 2015, **40**(2): 318-323.
[43] Song L, Li X, Bai XX, *et al*. Calycosin improves cognitive function in a transgenic mouse model of Alzheimer's disease by activating the protein kinase C pathway [J]. *Neural Regen Res*, 2017, **12**(11): 1870-1876.
[44] Li JJ, Cui GZ, Wang L, *et al*. Protective effects of calycosin on oxygen glucose deprivation-induced cell injury in H9c2 cell [J]. *Pharm Clini Chin Mater Medica*, 2014, **30**(05): 32-35.
[45] Dominic EA, Ramezani A, Anker SD, *et al*. Mitochondrial cytopathies and cardiovascular disease [J]. *Heart*, 2014, **100**(8): 611-618.
[46] Liu B, Zhang J, Liu W, *et al*. Calycosin inhibits oxidative stress-induced cardiomyocyte apoptosis *via* activating estrogen receptor-alpha/beta [J]. *Bioorg Med Chem Lett*, 2016, **26**(1): 181-185.
[47] Zhu H, Zhang Y, Ye G, *et al*. *In vivo* and *in vitro* antiviral activities of calycosin-7-*O*-beta-D-glucopyranoside against coxsackie virus B3 [J]. *Biol Pharm Bull*, 2009, **32**(1): 68-73.
[48] Moe GW, Marin-Garcia J. Role of cell death in the progression of heart failure [J]. *Heart Fail Rev*, 2016, **21**(2): 157-167.
[49] Ferrari R, Balla C, Malagu M, *et al*. Reperfusion damage-a story of success, failure, and hope [J]. *Circ J*, 2017, **81**(2): 131-141.
[50] Yang Q, He GW, Underwood MJ, *et al*. Cellular and molecular mechanisms of endothelial ischemia/reperfusion injury: perspectives and implications for postischemic myocardial protection [J]. *Am J Transl Res*, 2016, **8**(2): 765-777.
[51] Junqing G, Tao C, Huigen J, *et al*. Effect of calycosin on left ventricular ejection fraction and angiogenesis in rat models with myocardial infarction [J]. *J Tradit Chin Med*, 2015, **35**(2): 160-167.
[52] Ren M, Wang X, Du G, *et al*. Calycosin-7-O-beta-D-glucoside attenuates ischemia reperfusion injury *in vivo via* activation of the PI3K/Akt pathway [J]. *Mol Med Rep*, 2016, **13**(1): 633-640.
[53] Cheng Y, Zhao J, Tse HF, *et al*. Plant natural products calycosin and gallic acid synergistically attenuate neutrophil infiltration and subsequent injury in isoproterenol-induced myocardial infarction: a possible role for leukotriene B4 12-hydroxydehydrogenase? [J]. *Oxid Med Cell Longev*, 2015, **2015**: 434052.
[54] Li CJ, Xing XX, Zhou ZC, *et al*. Study of protective mechanism of calycosinon on myocardial ischemia-reperfusion injury in rats [J]. *Tianjin J Tradit Chin Med*, 2017, **34**(05): 341-344.
[55] Bao LT. The experimental study of calycosin's effect on cardiac hypertrophy in mice [D]. Wuhan: Wuhan University, 2017.
[56] Huang J, Cheng CF, Li HB, *et al*. The potential protect effect and mechanism of calycosin on cardiac hypertrophy in rats [J]. *The J Pract Med*, 2018, **34**(24): 4060-4063.
[57] Tang JY, Li S, Li ZH, *et al*. Calycosin promotes angiogenesis involving estrogen receptor and mitogen-activated protein kinase (MAPK) signaling pathway in zebrafish and HUVEC [J]. *PLoS One*, 2010, **5**(7): e11822.
[58] Wu XL, Wang YY, Cheng J, *et al*. Calcium channel blocking activity of calycosin, a major active component of Astragali radix, on rat aorta [J]. *Acta pharmacol Sin*, 2006, **27**(8): 1007-1012.
[59] Tseng HH, Vong CT, Leung GP, *et al*. Calycosin and formononetin induce endothelium-dependent vasodilation by the activation of large-conductance Ca(2+)-activated K(+) channels (BKCa) [J]. *Evid Based Complement Alternat Med*, 20165272531.
[60] Jiang YH, Sun W, Li W, *et al*. Calycosin-7-O-beta-D-glucoside promotes oxidative stress-induced cytoskeleton reorganization through integrin-linked kinase signaling pathway in vascular endothelial cells [J]. *BMC complement Altern Med*, 2015, **15**: 315.
[61] Li S, Dang YY, Oi Lam Che G, *et al*. VEGFR tyrosine kinase inhibitor II (VRI) induced vascular insufficiency in zebrafish as a model for studying vascular toxicity and vascular preservation [J]. *Toxicol Appl Pharmacol*, 2014, **280**(3): 408-420.
[62] Xu YH, Xiong J, Wang SS, *et al*. Calycosin entered HUVECs and ameliorated AGEs-promoted cell apoptosis *via* the Bcl-2 pathway [J]. *J Nat Med*, 2014, **68**(1): 163-172.
[63] Xu Y, Feng L, Wang S, *et al*. Phytoestrogen calycosin-7-O-beta-D-glucopyranoside ameliorates advanced glycation end products-induced HUVEC damage [J]. *J Cell Biochem*, 2011, **112**(10): 2953-2965.
[64] Li XL, Song RX, Lin X, *et al*. Comparison of calycosin and irbesartan for their impact on renin angiotensin system in human umbilical vein endothelial cell [J]. *Minerva Med*, 2015, **106**(1): 9-16.
[65] Tang BS. Inhibiting effects of calycosin on expression of ICAM-1 in vascular endothelial cell and its receptor LFA-1 [D]. Lanzhou: Lanzhou University, 2011.

[66] Wang XT. Effect of calycosin on the synthesis of PGI2 and TXA2 in vascular endothelial cells [D]. Lanzhou: Lanzhou University, 2011.
[67] Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis [J]. *Circ Res*, 2016, **119**(1): 91-112.
[68] Zhang YY, Tan RZ, Zhang XQ, *et al*. Calycosin ameliorates diabetes-induced renal inflammation *via* the NF-kappaB pathway *in vitro* and *in vivo* [J]. *Med Sci Monit*, 2019, **25**: 1671-1678.
[69] Quan GH, Wang H, Cao J, *et al*. Calycosin suppresses RANKL-mediated osteoclast-ogenesis through inhibition of MAPKs and NF-kappaB [J]. *Int J Mol Sci*, 2015, **16**(12): 29496-29507.
[70] Dong L, Yin L, Chen R, *et al*. Anti-inflammatory effect of Calycosin glycoside on lipopolysaccharide-induced inflammatory responses in RAW 264.7 cells [J]. *Gene*, 2018, **675**: 91-101.
[71] Jaremo P, Eriksson-Franzen M, Milovanovic M. Platelets, gender and acute cerebral infarction [J]. *J Transl Med*, 2015, **13**: 267.
[72] Russo I, Penna C, Musso T, *et al*. Platelets, diabetes and myocardial ischemia/reperfusion injury [J]. *Cardiovasc Diabetol*, 2017, **16**(1): 71.
[73] Liao F, Yu A, Yu J, *et al*. Identification of active ingredients mediating anti-platelet aggregation effects of BuyangHuanwu decoction using a platelet binding assay, solid phase extraction, and HPLC-MS/MS [J]. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2018, **1092**: 320-327.
[74] Liu H, Liang JP, Li PB, *et al*. Core bioactive components promoting blood circulation in the traditional Chinese medicine compound xueshuantong capsule (CXC) based on the relevance analysis between chemical HPLC fingerprint and *in vivo* biological effects [J]. *PLoS One*, 2014, **9**(11): e112675.
[75] Dong TT, Zhao KJ, Gao QT, *et al*. Chemical and biological assessment of a chinese herbal decoction containing radix Astragali and radix Angelicae Sinensis: determination of drug ratio in having optimized properties [J]. *J Agric Food Chem*, 2006, **54**(7): 2767-2774.
[76] Sheng S, Wang J, Wang L, *et al*. Network pharmacology analyses of the antithrombotic pharmacological mechanism of Fufang Xueshuantong Capsule with experimental support using disseminated intravascular coagulation rats [J]. *J Ethnopharmacol*, 2014, **154**(3): 735-744.
[77] Yu DH, Bao YM, Wei CL, *et al*. Studies of chemical constituents and their antioxidant activities from *Astragalus mongholicus Bunge* [J]. *Biomed Environ Sci*, 2005, **18**(5): 297-301.
[78] Li S, Wang Y, Feng C, *et al*. Calycosin inhibits the migration and invasion of human breast cancer cells by down-regulation of Foxp3 expression [J]. *Cell Physiol Biochem*, 2017, **44**(5): 1775-1784.

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