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Research Progress of Astragaloside IV in the Treatment of Cardiovascular Diseases

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Abstract: Cardiovascular disease (CVD) continues to dominate as the primary cause of mortality and morbidity worldwide, constituting a pressing global health concern. In contrast to Western medicine, traditional Chinese medicine (TCM) offers a holistic, side-effect-minimizing, and highly efficacious approach to tackling CVD challenges. Among the myriad herbs utilized in TCM, Huangqi (HQ), particularly in the realm of cardiovascular therapeutics, has enjoyed an esteemed status spanning millennia. Astragaloside IV (AS-IV), a saponin derivative meticulously extracted from the roots of the renowned Chinese medicinal plant *Astragalus membranaceus*, has garnered significant attention for its multifaceted cardioprotective capabilities. These encompass antioxidant stress mitigation, anti-inflammatory actions, anti-apoptotic effects, inhibition of cardiomyocyte hypertrophy, and attenuation of myocardial fibrosis, among others. Consequently, pharmacokinetic and toxicological evaluations underscore AS-IV's low bioavailability yet commendable safety profile, with a notable caveat of prudence when administering to pregnant individuals. The present article delves into the most recent advancements in understanding the therapeutic impacts and underlying mechanisms of AS-IV in the context of cardiovascular diseases. By consolidating these cutting-edge findings, we aspire to establish a robust theoretical foundation that can propel the development of AS-IV as an innovative therapeutic agent for the treatment of CVDs, thereby contributing to the global endeavor to combat this pervasive health burden.

Keywords: Astragaloside IV; Cardiovascular diseases; Pharmacology

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1. Introduction

Cardiovascular disease (CVD) stands as the foremost contributor to mortality in China's population health, earning it the sobriquet of the "number one killer." Annually, approximately one million lives are lost to CVD, constituting roughly 45% of the country's total mortality rate, posing a dire threat to human wellbeing. Amidst this escalating burden of CVD, relentless efforts have been directed toward discovering novel drugs and therapeutic strategies. Undeniably, CVD has transformed into a pressing public health crisis. The groundbreaking discovery of artemisinin by Tu Youyou underscores the immense potential harbored within traditional Chinese medicine (TCM).

Over the past few decades, natural compounds derived from Chinese herbal medicines have emerged as invaluable resources for drug research and development, particularly in the realm of CVD treatment. These compounds offer promising avenues for addressing the challenges posed by CVD and represent a significant advancement in the quest for innovative therapeutic solutions [1].

Astragaloside IV (AS-IV), a pivotal constituent extracted from *Astragalus membranaceus* var *mongholicus* (commonly known as Huangqi, HQ, in China), boasts a rich history as a fundamental Qi-tonifying medicine renowned for its broad therapeutic spectrum. Frequently employed in the prevention and management of heart diseases [2]. HQ is characterized by its gentle yet potent actions, diverse therapeutic targets, minimal toxic side effects, cost-effectiveness, and overall safety. Its chemical makeup encompasses a diverse array of glycosides, polysaccharides, amino acids, and trace elements, collectively contributing to its status as a natural antioxidant. Notably, HQ's therapeutic prowess stems from three primary ingredients: HQ saponins, HQ polysaccharides, and HQ flavonoids, with AS-IV occupying a prominent position among the saponin fraction. Possessing poor water solubility yet readily soluble in ethanol, AS-IV carries a molecular formula of $C_{41}H_{68}O_{14}$ and a molecular weight of 784.98. Its medicinal scope extends across multiple physiological systems, encompassing the digestive, nervous, endocrine, respiratory, urinary, hematological, and cardiovascular systems. Within the realm of cardiovascular medicine, AS-IV has garnered extensive research attention, particularly in the treatment of cardiovascular diseases (CVDs), yielding notable advancements that pave the way for novel drug development. This article comprehensively summarizes the cardiovascular pharmacological activities of AS-IV, delving into its therapeutic effects and underlying mechanisms in addressing CVDs. By consolidating the latest findings, the study aims to provide a comprehensive overview of AS-IV's potential as a therapeutic agent, fostering further exploration and innovation in the field of cardiovascular therapeutics.

AS-IV, a naturally occurring compound meticulously extracted and purified from the medicinal herb *Astragalus membranaceus*, exhibits a versatile array of pharmacological properties, including potent anti-inflammatory [3], antioxidant [4], anti-myocardial hypertrophic [5], anti-myocardial apoptotic [6], and anti-myocardial fibrotic activities [7] (**Figure 1**). Its burgeoning application in the cardiovascular domain is steadily yielding groundbreaking advancements. Despite these promising prospects, the current research landscape surrounding AS-IV remains relatively circumscribed. To gain a deeper insight into the progress made in elucidating AS-IV's therapeutic potential for cardiovascular diseases (CVDs), we embarked on a comprehensive review of the systematic studies conducted on AS-IV in recent years. This endeavor aimed to provide a consolidated and up-to-date summary of the research advancements, thereby facilitating further exploration and advancing the field of cardiovascular therapeutics.

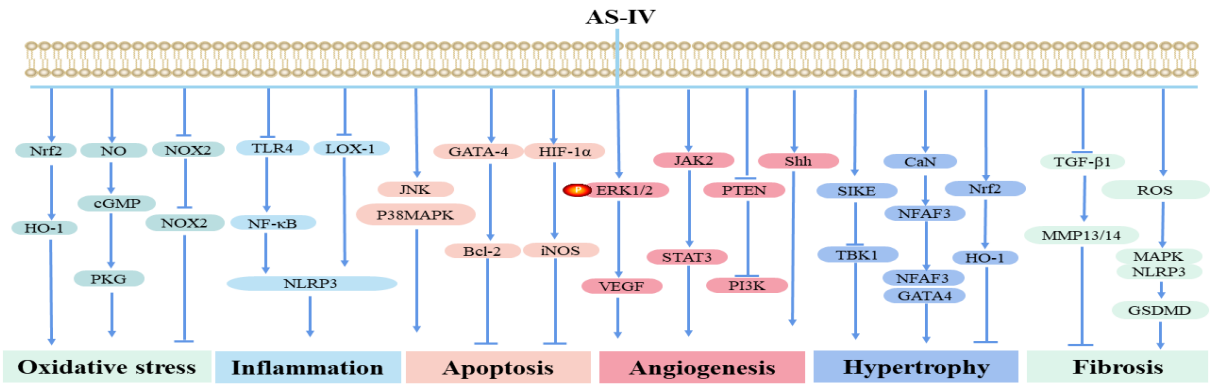

Figure 1. Pharmacological effects of AS-IV on myocardium. AS-IV has protective effects such as anti-cardiomyocyte hypertrophy, anti-fibrosis, antioxidant stress, anti-inflammatory, anti-apoptosis, and promotion of angiogenesis.

2. Chemical structure and pharmacological properties of AS-IV

Astragaloside IV (CHEBI: 65457 Astragaloside IV) (chemical structure presented in **Figure 2**) is a pentacyclic triterpenoid that is cycloastragenol having beta-D-xylopyranosyl and beta-D-glucopyranosyl residues attached at positions O-3 and O-6 respectively. It has a role as an EC 4.2.1.1 (carbonic anhydrase) inhibitor, an anti-inflammatory agent, a neuroprotective agent, an antioxidant, a pro-angiogenic agent and a plant metabolite. It is a triterpenoid saponin and a pentacyclic triterpenoid. It is functionally related to a cycloastragenol. The detailed physicochemical properties of AS-IV are summarized in **Table 1**.

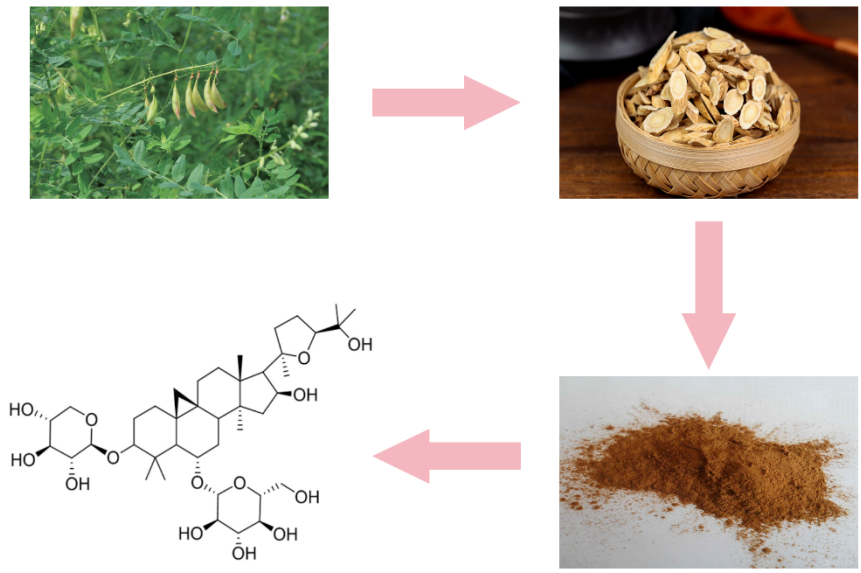

Figure 2. Structure and origin of AS-IV. AS-IV is a pentacyclic triterpenoid that is cycloastragenol having β-D-xylopyranosyl and β-D-glucopyranosyl residues attached at positions O-3 and O-6 respectively. The detailed physicochemical properties of AS-IV was sourced from CHEBI.

Table 1. Physicochemical properties of Astragaloside IV

Content	Description
Name	Astragaloside IV
Molecular formula	$C_{41}H_{68}O_{14}$
Another name in English	(3β,6α,9β,16β,20R,24S)-16,25-Dihydroxy-3-(β-D-xylopyranosyloxy)-20,24-epoxy-9,19-cyclolanostan-6-yl β-D-glucopyranoside
Molecular weight	785.0 g/mol
PSA	228.22000
LogP	1.96
Boiling point	895.7 ± 65.0 °C at 760 mmHg
Melting point	295–296 °C
Appearance traits	White crystalline powder
Molecular weight	784.970 g/mol
Flash point	495.5 ± 34.3 °C
Refractive index	1.621
Storage conditions	2–8°C
Density	1.4 ± 0.1 g/cm^3

3. Potential pharmacological effects of Astragaloside IV on cardiovascular diseases

3.1. Inhibit oxidative stress

Oxidative stress represents an intricate imbalance within the physiological milieu, characterized by a heightened presence of oxidants and a concurrent decline in antioxidants. This state is particularly marked by the pivotal role of reactive oxygen species (ROS), whose excessive generation, when coupled with nitric oxide (NO) to yield peroxynitrite, diminishes NO's biological efficacy and precipitates endothelial dysfunction. Myocardial ischemia/reperfusion (I/R) injury poses a formidable clinical challenge, as the initial moments of reperfusion following ischemia precipitate a surge of free radicals, which, coupled with reduced antioxidant defenses, renders the myocardium acutely susceptible to damage [8]. Succinic acid, a vital intermediate in the tricarboxylic acid (TCA) cycle, has been implicated in the amplification of ROS production during I/R processes [9]. Notably, Jiang *et al.* (2019) demonstrated that AS-IV, administered at a dose of 40 mg/kg, effectively mitigated the accumulation of succinic acid in the myocardium of Sprague-Dawley (SD) rats subjected to I/R, subsequently attenuating ROS generation. Furthermore, AS-IV activates the Nrf2/HO-1 signaling cascade, upregulating the expression of antioxidant enzymes, thereby conferring a cardioprotective benefit [10]. Glycogen synthase kinase-3β (GSK-3β) emerges as a central regulator in the orchestration of cellular apoptosis [11]. In this context, He *et al.* (2012) revealed that AS-IV, at a concentration of 50 μM, inhibits oxidative stress-mediated mitochondrial permeability transition pore (mPTP) opening in H9C2 cells through the NO/cGMP/PKG/GSK-3β signaling pathway [12]. Additionally, AS-IV, administered at doses of 5 and 10 mg/kg, mitigated myocardial I/R injury in SD rats by modulating the PI3K/Akt pathway, leading to GSK-3β phosphorylation, further underscoring its cardioprotective potential [13].

Doxorubicin (DOX), a cornerstone of anti-tumor chemotherapy, is notorious for its potential to inflict severe cardiac toxicity. This toxicity is underpinned by DOX's ability to elicit oxidative stress, a process facilitated by the upregulation of NADPH oxidase isoforms NOX2 and NOX4 in rat hearts, ultimately culminating in cardiomyopathy [14]. Lin *et al.* (2019) seminally reported that AS-IV, administered at a dose of 40 mg/kg, effectively mitigated myocardial injury, apoptosis, fibrosis, and dysfunction in C57BL/6 mice exposed to DOX, achieving this by suppressing the expression of NOX2 and NOX4 [15]. Furthermore, AS-IV has been shown to bolster the myocardial antioxidant defense system, as evidenced by its enhancement of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and catalase (CAT) activities in both mouse models of viral myocarditis and rat models of DOX-induced heart injury. Concurrently, AS-IV reduced the levels of myeloperoxidase (MPO) and reactive oxygen species (ROS), thereby alleviating oxidative stress [15,16]. Calpain-1, a protease present in endothelial cells, has been implicated in the pathogenesis of endothelial dysfunction [17]. Nie *et al.* (2019) interestingly discovered that AS-IV could ameliorate endothelial dysfunction in the thoracic aorta of diabetic rats by dual mechanisms, decreasing oxidative stress and inhibiting Calpain-1 activity [18]. This finding underscores the multifaceted cardioprotective effects of AS-IV and highlights its potential as a therapeutic strategy for mitigating the adverse cardiac effects of DOX and other oxidative stress-mediated conditions.

3.2. Anti-inflammatory

Inflammation stands as a pivotal and independent cardiovascular risk factor, with the capacity to not only inflict endothelial damage but also contribute significantly to the pathogenesis of endothelial dysfunction [19]. In the inflammatory cascade, cellular inflammatory factors adhere to and aggregate on endothelial cells, triggering the release of noxious metabolites from vascular endothelial cells. This process disrupts the structural and functional integrity of the endothelium, thereby precipitating endothelial dysfunction [20]. By understanding these intricate mechanisms, more insights can be gained into the role of inflammation in cardiovascular health and devise strategies to mitigate its adverse effects.

AS-IV glycoside exerts its anti-inflammatory effects by disrupting the inflammatory signaling pathway,

thereby mitigating the deleterious impact of the inflammatory response on endothelial cells. The expression of adhesion molecules, notably vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), on the endothelial cell surface plays a pivotal role in the initiation and progression of inflammation. In the presence of inflammation, these adhesion molecules facilitate the adherence of white blood cells to endothelial cells [21]. Notably, the TLR4/NF-κB signaling pathway regulates the expression of VCAM-1 and ICAM-1, underscoring its importance in the inflammatory process [22]. AS-IV demonstrates efficacy in ameliorating vascular endothelial dysfunction associated with hyperglycemia. It achieves this by reducing elevated levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), while concurrently decreasing the expression of VCAM-1 and ICAM-1 [23]. The protective effects of AS-IV may be mediated, at least in part, through the TLR4/NF-κB signaling pathway. In the context of diabetic angiopathy, the accumulation of oxidized low-density lipoprotein (ox-LDL) exacerbates endothelial cell injury through a multifaceted mechanism involving increased oxidative stress, augmented inflammatory response, and heightened secretion of adhesion molecules [24]. Ox-LDL triggers inflammation by stimulating the production of numerous inflammatory cytokines, including IL-β, whose maturation is orchestrated by the NLRP3 inflammasome. Additionally, ox-LDL activates the NLRP3 inflammasome, leading to the secretion of IL-1β in macrophages [25]. Remarkably, AS-IV safeguards endothelial progenitor cells (EPCs) from oxLDL-induced dysfunction by targeting the LOX-1/NLRP3 signaling pathway [26].

Toll-like receptor 4 (TLR4) stands as a pivotal LPS receptor and a crucial mediator of proinflammatory responses. Zhang *et al.* (2019) have demonstrated that AS-IV, administered at doses of 20, 40, and 80 mg/kg, significantly ameliorated heart function, myocardial cell viability, and pathological alterations elicited by lipopolysaccharide (LPS) exposure in C57BL/6J mice. Furthermore, AS-IV effectively reduced the concentrations of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) within myocardial tissue [27]. In a separate study, Yang *et al.* (2013) induced a myocardial hypertrophy model through intraperitoneal injections of isoproterenol (5 mg/kg/d) and observed that AS-IV, at doses of 20, 40, and 80 mg/kg, inhibited myocardial hypertrophy and reduced serum levels of TNF-α and IL-6. Their findings suggested that this protective effect was associated with the inhibition of the TLR4/NF-κB signaling pathway [28]. Shi *et al.* (2021) further elaborated on the therapeutic potential of AS-IV in myocardial inflammation, reporting that AS-IV (80 mg/kg/day) alleviated myocarditis in SD rats with acute myocardial infarction (AMI) by suppressing the TLR4/MyD88/NF-κB pathway [29]. Extending the investigation to sepsis-induced cardiac dysfunction, Huang *et al.* (2021) examined the effects of AS-IV on rats subjected to cecal ligation and puncture (CLP). They found that AS-IV (40 mg/kg/d) mitigated myocardial cell apoptosis post-CLP surgery and reduced serum levels of inflammatory markers, including IL-6, IL-10, and high mobility group box 1 (HMGB-1) [30]. Collectively, these studies underscore the multifaceted protective roles of AS-IV in mitigating cardiac dysfunction and inflammation, particularly through its modulation of the TLR4/NF-κB signaling pathway and its downstream inflammatory cascades.

3.3. Anti-myocardial apoptosis

Apoptosis holds a pivotal role in numerous tissue cell types, and its regulation is orchestrated by a complex network of genes. Notably, the *Bcl-2* gene family, which was the first to be identified as a key regulator of apoptosis, comprises members such as *Bcl-2*, *Bax*, *Bcl-XS*, and *Bcl-XL*, with some promoting while others inhibiting apoptosis [31]. Prior research has established that the delicate balance of *Bcl-2/Bax* expression ratios in cardiac myocytes during both physiological and pathological processes dictates cell fate, either fostering survival or inducing death [32]. Furthermore, evidence suggests that AS-IV exerts a protective effect in myocardial injury scenarios, specifically by downregulating *Bax* expression and upregulating *Bcl-2* expression. This modulation results in an elevated *Bcl-2/Bax* ratio, ultimately inhibiting myocardial cell apoptosis and mitigating myocardial

damage following doxorubicin-induced injury [33]. In an experimental model involving intraperitoneal inoculation of Coxsackievirus B3 in *Balb/c* mice, AS-IV significantly reduced the apoptosis index of compromised myocardial cells, thereby showcasing its potent anti-apoptotic properties. This finding further underscores AS-IV's ability to indirectly delay or even reverse myocardial fibrosis [34]. The apoptosis of myocardial cells is intricately tied to the expression of apoptotic genes. AS-IV's therapeutic mechanism involves inhibiting the expression of various genes that promote myocardial cell apoptosis, thereby suppressing cell death and preserving cardiac function.

The MAPK signaling cascade plays a crucial role in modulating cell apoptosis, encompassing three distinct kinase families: c-Jun N-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs), and p38 mitogen-activated protein kinases (p38 MAPKs) [35,36]. Sun *et al.* (2021) demonstrated that AS-IV, at concentrations of 10 or 50 ng/mL, effectively safeguards H9C2 cells against apoptosis triggered by high glucose/high fat (HG/HF) conditions and hypoxia. This protective effect is attributed to its ability to dampen the activation of JNK and p38 signaling pathways while fostering the activation of the ERK signaling pathway [37]. In animal studies, AS-IV administration at doses of 10 and 50 mg/kg/day to C57BL/6 mice mitigated cardiac dysfunction induced by streptozotocin (STZ) through fine-tuning the MAPK signaling pathway. This intervention not only inhibited myocardial fibrosis and inflammation but also preserved cardiac function [37]. Calpain-1, a member of the cysteine protease family, has been implicated in promoting cell apoptosis during myocardial ischemia/reperfusion (I/R) and pressure/overload conditions [38,39]. Mei *et al.* (2015) reported that AS-IV, administered at 80 mg/kg/day, attenuated isoproterenol-induced apoptosis in hypertrophic cardiomyocytes of Sprague-Dawley (SD) rats by inhibiting calpain-1 activity and mitigating oxidative stress [40]. Moreover, Yang *et al.* (2020) observed that AS-IV, within a concentration range of 0.5–300 μg/mL, promoted *Bcl-2* expression in H9c2 cells by stimulating the overexpression of GATA-4. This upregulation led to a reduction in apoptosis induced by hypoxia/reoxygenation (H/R) [41]. Hypoxia-inducible factor-1α (HIF-1α) serves as a pivotal regulator in the molecular response to hypoxia, enhancing oxygen transport and facilitating metabolic adaptation to hypoxic conditions through the activation of genes related to energy metabolism, angiogenesis, and cell apoptosis [42]. Si *et al.* (2014) discovered that AS-IV, at a concentration of 50 μM, upregulated the HIF-1α/iNOS signaling pathway in rat neonatal cardiomyocytes (RNCM), leading to increased cell viability post-ischemia. This effect was accompanied by a decrease in the apoptosis index and lactate dehydrogenase (LDH) release, indicating reduced cellular damage [43].

3.4. Promote angiogenesis

Angiogenesis, a pivotal pathological event in a myriad of chronic ischemic diseases, necessitates strategic reconstruction within ischemic regions as a paramount approach to enhancing disease prognosis [44]. Endothelial cells, the cornerstone of vascular endothelium, are instrumental in orchestrating angiogenesis, extending from the heart to the finest microvasculature. Vascular endothelial growth factor (VEGF), a crucial regulator, propels blood vessel formation. Studies have illuminated that AS-IV fosters angiogenesis through elevating VEGF and basic fibroblast growth factor (bFGF) levels. Wang *et al.* (2015) solidified this notion by demonstrating that AS-IV augments human umbilical vein endothelial cell (HUVEC) proliferation and angiogenesis via the ERK1/2 pathway activation, thereby modulating VEGF production [45]. Notably, ERK1/2 occupies a pivotal position in the angiogenesis cascade [46]. Furthermore, their investigation revealed that AS-IV, at concentrations of 10, 40, and 120 μM, accentuates HUVEC proliferation, migration, and tubular formation mechanisms by upregulating ERK1/2 phosphorylation and engaging the JAK2/STAT3 pathway [47]. In addition to these findings, Zhang *et al.* (2012) contributed to the understanding by uncovering that AS-IV, specifically at concentrations of 10 μg/mL and 100 μg/mL, promotes angiogenesis in HUVECs through the Akt pathway activation [48].

Furthermore, the overexpression of phosphatase and tensin homolog (*PTEN*) deleted on chromosome ten,

a gene absent from the human chromosome, is implicated in eliciting endothelial dysfunction, a condition that predisposes thrombosis. Conversely, downregulation of *PTEN* expression fosters VEGF expression, subsequently stimulating angiogenesis via potentiation of VEGF-mediated signal transduction in target cells [49]. Cheng *et al.* (2019) demonstrated that AS-IV exerts a reparative effect on cardiac function post-myocardial infarction, accompanied by heightened survival rates, diminished infarct sizes, amelioration of pathological alterations and fibrosis, as well as augmented angiogenesis, thereby corroborating AS-IV's angiogenic and cardioprotective properties post-infarction. This salutary effect is mediated, in part, through the PTEN/PI3K/Akt signaling cascade [50]. Moreover, STAT3 occupies a pivotal position in angiogenesis within the context of cardiac pathogenesis, positioning it as a promising molecular target for angiogenesis-targeted therapeutic strategies [51]. Sui *et al.* (2019) revealed that AS-IV stimulates angiogenesis in SD rats subjected to left coronary artery ligation-induced heart failure via activation of the JAK-STAT3 signaling pathway [52]. Connexin (Cx), a family of structurally interdependent transmembrane proteins, facilitates the formation of gap junctions, essential for intercellular communication. Notably, Cx37, Cx40, and Cx43 are intimately linked to the angiogenic process [53–55]. Li *et al.* (2018) discovered that AS-IV at a concentration of 0.2 μg/mL enhances gap junction intercellular communication by upregulating the expression of Cx37, Cx40, and Cx43, ultimately facilitating endothelial cell angiogenesis [56].

Sonic hedgehog (Shh) is a crucial regulator for maintaining the integrity of the coronary vascular system and acts as a potent pro-angiogenic factor in the context of ischemic diseases. Consequently, Shh emerges as a promising therapeutic target in the management of myocardial infarction. Wang *et al.* (2017) have demonstrated that both tetramethylpyrazine (TMP) and Astragaloside IV (AS-IV), either administered individually or in combination, effectively enhance left ventricular remodeling and safeguard cardiac function in a rat model of myocardial infarction. The underlying mechanism may involve the upregulation of signaling molecules associated with the Shh pathway, thereby triggering cardiac angiogenesis, as a pivotal step in their cardioprotective effects [57].

3.5. Anti-myocardial hypertrophy

Myocardial hypertrophy (MH) represents the heart's compensatory response to various pathological stimuli, enabling it to maintain adequate systolic function. However, prolonged MH can culminate in myocardial ischemia, arrhythmias, heart failure, and even sudden death, thereby emerging as an independent risk factor that significantly escalates the incidence and mortality rates associated with cardiovascular diseases [58,59]. Utilizing a mouse model of MH induced by subcutaneous isoproterenol injection, the study observed a marked increase in heart weight index (16.4%) and left heart index (24.2%) post-injection. Notably, AS-IV administration significantly mitigated these indices, indicating its potential to inhibit isoproterenol-induced MH, particularly during the early stages of heart failure or cardiac functional compensation [28]. Furthermore, in a model of left ventricular hypertrophy (LVH) induced by pressure overload, AS-IV demonstrated its efficacy in reducing LV mass index, plasma angiotensin II (Ang II), and aldosterone levels, along with Ang II concentrations in myocardial tissue [60]. This suggests that AS-IV modulates Ang II levels in both plasma and myocardial tissue, diminishes aldosterone in plasma, downregulates *ACE* gene expression, and upregulates *AT2* gene and protein expression, thereby reversing LVH. However, it did not impact *AT1* gene expression. AS-IV's ability to inhibit the overactivation of the renin-angiotensin system in pressure overload-induced MH rats underscores a potential pathway for its LVH-reversing effects. TBK1, also known as NF-κB activated kinase, promotes NF-κB translocation, leading to inflammation [61]. Liu *et al.* (2014) reported that AS-IV (10 and 20 mg/kg/day) attenuated MH, inflammatory responses, and cardiomyocyte apoptosis induced by aortic valve stenosis in C57BL6 mice. This was likely mediated through SIKE upregulation and inhibition of TBK1/PI3K/Akt activity. Additionally, CaN, a calcium-activated serine/threonine protein phosphatase, interacts with NFAT-3 transcription factors, facilitating their nuclear translocation

and complex formation with GATA-4, contributing to MH [62]. The transcription factor Nrf2, a key regulator of cellular antioxidant defenses, was found to be upregulated by AS-IV (40 and 80 mg/kg/day) in AAC-induced rat MH models, implicating the Nrf2/HO-1 signaling pathway in its mechanism of action [63]. *In vitro* studies using neonatal rat cardiomyocytes pretreated with AS-IV (30 μmol/L) effectively countered MH-related total protein volume increases and ANP gene expression, underscoring its protective role [64]. Moreover, AS-IV inhibited ISO-mediated IκBα degradation, thereby blocking TLR4/NF-κB signaling activation and suppressing MH progression. These findings collectively highlight AS-IV's multifaceted mechanisms in mitigating MH, encompassing regulation of the renin-angiotensin system, inflammatory pathways, and antioxidant defenses.

3.6. Anti myocardial fibrosis

Under pathological circumstances, such as inflammation and oxidative stress, cardiac fibroblasts (CFs) undergo differentiation into myofibroblasts, concurrently leading to excessive accumulation of extracellular matrix (ECM) [65]. Myocardial fibrosis (MF), characterized by the disproportionate deposition of collagen fibers within the myocardium, represents a pivotal pathological hallmark in the progression of heart failure. This phenomenon directly impairs myocardial compliance and diminishes systolic function, potentially precipitating arrhythmias, exacerbating cardiac pump failure, and even culminating in sudden cardiac death. Matrix metalloproteinases (MMPs), the primary enzymatic system responsible for ECM degradation, are counterbalanced by their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Intriguingly, a study investigating mice with chronic myocarditis and MF revealed a substantial reduction in heart failure incidence and mortality rates among those treated with astragalus saponins. Further research underscores that astragalus saponins exert their beneficial effects by attenuating the expression of transforming growth factor-β1 (TGF-β1) while enhancing the expression of MMP-13 and MMP-14, thereby mitigating myocardial fibrosis in chronic myocarditis models [63]. This suggests that astragalus saponins' anti-fibrotic effects may stem from inhibiting TGF-β1 signaling and upregulating MMP activity in myocardial tissue. Moreover, *in vivo* studies demonstrate that Astragaloside IV (AS-IV) protects myocardial function and tissue integrity in rats subjected to high-dose isoproterenol (Iso)-induced myocardial hypertrophy, achieved through intraperitoneal injection of 5 mg/kg/day Iso [66]. Notably, oxidative stress is intimately linked to myocardial fibrosis, and Dai *et al.* (2017) discovered that AS-IV (100 μM) mitigates Iso-induced cardiac fibrosis by suppressing reactive oxygen species (ROS)-mediated MAPK activation [67]. Inflammation is another pivotal player in the progression of cardiac fibrosis [68]. The NLRP3 inflammasome, a critical regulator of inflammatory responses, promotes the maturation and release of pro-inflammatory cytokines like IL-18 and IL-1β [69]. Wan *et al.* (2018) reported that AS-IV (200 mg/kg/day) alleviates Iso-induced cardiac fibrosis in BALB/c mice by inhibiting the NLRP3 inflammasome pathway [70]. Additionally, Zhang *et al.* (2022) observed that AS-IV (40 mg/kg/day) ameliorates myocardial fibrosis and hypertrophy in C57BL/6J mice with acute myocardial infarction, a mechanism attributed to the inhibition of the ROS/NLRP3/GSDMD signaling cascade, ultimately reducing apoptosis [71].

3.7. Anti-arrhythmic

Arrhythmia encompasses abnormalities in the origination of heart rhythm, the frequency and pattern of new beats, as well as impulse conduction, with a heightened prevalence observed in diverse organic cardiovascular disorders. Studies have demonstrated that HQ injection can postpone the impact of digitalis on arrhythmia by enhancing the functionality of Na^+-K^+-ATPase in myocardial tissue that is under inhibition [72]. Furthermore, AS-IV has been shown to markedly suppress the elongation of QRS duration and the augmentation of T wave amplitude, both of which are induced by toad venom in mice. This effect not only decreases the incidence of ventricular arrhythmias

but also significantly extends survival time, thereby exerting a protective role against toad venom-induced ventricular arrhythmias [73].

4. The effects of Astragaloside IV on hemodynamics and toxicology

Research has conclusively established that AS-IV injection does not exert a noteworthy influence on partial thromboplastin time, blood enzyme time, or thrombin time in rabbits [74]. Although AS-IV injection effectively diminishes whole blood viscosity in rabbits, its efficacious dosage range is comparatively constrained. Moreover, while AS-IV glycoside alters blood flow rheology in rabbits, this effect is moderately weak. Zhang *et al.* (2006) observed that over 83% of AS-IV binds to plasma proteins, showcasing a linear correlation within a concentration range of 250–1000 ng/mL [75]. Notably, the elimination half-lives of AS-IV in male SD rats (administered at doses of 0.75, 1.5, and 3.0 mg/kg) were 98.1, 67.2, and 71.8 minutes, respectively, whereas, in female SD rats, they were 34.0, 66.9, and 131.6 minutes, respectively. Interestingly, there was no marked difference in the systemic clearance rate of AS-IV, indicating its potential for linear pharmacokinetic behavior within the experimental dosage range. Consistent with this, AS-IV exhibited linear pharmacokinetic characteristics in beagle dogs as well, with elimination half-lives of 51.9, 60.0, and 68.8 minutes in males and 62.9, 67.2, and 50.2 minutes in females at doses of 0.25, 0.5, and 1 mg/kg, respectively. The tissue distribution of AS-IV reveals a preferential accumulation in the lungs and liver, whereas its penetration into the brain is limited, possibly owing to the challenge of traversing the blood-brain barrier. Furthermore, 80% of AS-IV binds to serum proteins, and hepatic clearance is extremely slow, estimated at approximately 0.0041 kg/min. Zhang *et al.* (2006) delved into the excretion patterns of AS-IV following intravenous administration of 1.5 mg/kg in rats, uncovering that the total excretion through urine and feces in male rats amounted to 45.03% and 53.61%, respectively, indicating that approximately half of AS-IV undergoes *in vivo* metabolism [75]. Additionally, in mice and dogs, AST IV demonstrated a moderate to rapid clearance rate, maintaining linear kinetic properties within the dosage range of 0.75–3.0 mg/kg [75].

Gu *et al.* (2004) conducted a comprehensive evaluation of the transport mechanisms and bioavailability of AS-IV [76]. In mouse models, oral administration of AS-IV yielded suboptimal absorption, evidenced by absolute bioavailabilities of merely 3.66% and 2.2% across two distinct experimental settings [76,77]. An *in vitro* study utilizing Caco-2 cells elucidated that the poor absorption of AS-IV primarily stems from its high molecular weight, low lipophilicity, and reliance on paracellular transport pathways [78]. Zhang *et al.* (2007) similarly reported a modest absolute bioavailability of approximately 7.4% for AS-IV in beagle dogs [79]. *In vivo* investigations further corroborated that AS-IV undergoes absorption primarily through passive diffusion mechanisms [80]. Within a concentration range of 250 to 1000 ng/mL, the plasma protein binding rate of AS-IV approximates 90%. Nonetheless, the inherently low bioavailability of AS-IV poses significant limitations to its oral administration. To mitigate this challenge and enhance the oral bioavailability of AS-IV, the strategic design of its dosage forms is paramount. Several approaches have been proposed, including the utilization of chitosan, sodium deoxycholate, and AS-IV hydroxypropyl-β-cyclodextrin inclusion complexes, all of which have demonstrated potential to augment AS-IV absorption [78,81].

Yu *et al.* (2007) conducted a subchronic toxicity study on *Astragalus membranaceus* extract (RAE) in SD rats and beagle dogs [82]. Their findings indicate that RAE is deemed safe, without eliciting significant toxic side effects. Specifically, the safe dose range for SD rats was determined to be 5.7–39.9 g/kg, while for beagle dogs, it ranged from 2.85–19.95 g/kg, representing a 70- to 35-fold higher dose compared to the safe dose for humans. Additionally, the study explored the impact of AS-IV on embryonic development in rats and New Zealand white rabbits. In pregnant mice, a dose of 1.0 mg/kg AS-IV exhibited maternal toxicity, and doses exceeding 0.5 mg/kg

displayed embryotoxicity. However, no noteworthy visceral abnormalities or skeletal deformations were noted in either rats or New Zealand white rabbits [83].

Wan *et al.* (2010) conducted a comprehensive evaluation of the perinatal reproductive toxicity of AS-IV in SD rats, which did not uncover any clinical toxicity symptoms related to AS-IV in either male or female F0 rats throughout the pre-mating phase, the mating process, or during female pregnancy [84]. Furthermore, no notable differences were detected in the liver, kidneys, or reproductive organs of these rats. Following this, the study delved into the impact of AS-IV on the physiological and reflex development of F1 rats. Notably, a maternal dosage of 1.0 mg/kg/day of AS-IV significantly protracted the timelines for hair emergence, eye-opening, motor activity, and the cliff avoidance reflex. Conversely, memory and learning assessments failed to yield significant differences between the two groups.

In light of the anticipated human clinical dose of 10 mg/60 kg/day, it is paramount to gain a comprehensive understanding of the toxicity profile of AS-IV before its widespread clinical utilization. As a highly promising contender for novel drug development, further investigation is imperative to precisely delineate the effective and toxic dose ranges of AS-IV, taking into account its diverse therapeutic potential.

5. Conclusion and perspective

In summary, AS-IV demonstrates efficacy in ameliorating myocardial injury by mitigating oxidative stress, suppressing inflammatory responses and cell apoptosis, inhibiting myocardial cell hypertrophy, preventing myocardial fibrosis, and fostering angiogenesis. Furthermore, the multi-faceted and multi-target pharmacological effects of AS-IV glycoside align well with the intricate pathogenesis of various diseases, positioning it as a promising clinical candidate for the treatment of diseases and their associated complications. However, the development and clinical application of AS-IV as a novel drug face several challenges, primarily stemming from the complexity of its mechanism in treating cardiovascular diseases and the intricate network of its protective pathways. For instance, AS-IV (100μM) has been shown to hinder isoproterenol-induced myocardial fibrosis by inhibiting ROS-mediated MAPK activation, highlighting its ability to combat myocardial fibrosis through oxidative stress reduction [67]. Additionally, Liu *et al.* (2018) discovered that AS-IV (10 and 20mg/kg/day) mitigated myocardial hypertrophy, inflammatory responses, and cardiomyocyte apoptosis in C57BL6 mice by inhibiting the TBK1/PI3K/Akt pathway [85]. Meanwhile, AS-IV (40 and 80mg/kg/day) inhibited AAC-induced myocardial hypertrophy by upregulating the Nrf2/HO1 signaling pathway, indicating its antioxidant-mediated improvement of myocardial hypertrophy [86]. Zhang *et al.* (2021) further revealed that AS-IV (12.5 and 50 μM) protects HL-1 mouse cardiomyocytes from ox-LDL-induced oxidative damage by inhibiting HDAC activity [87]. These findings underscore the potential of AS-IV as a therapeutic agent but also emphasize the need for further research to fully elucidate its mechanisms of action and optimize its clinical application.

Mechanistically, researchers have broadened their scope beyond the examination of traditional cellular signaling pathways to delve into the role of non-coding RNAs, particularly microRNAs (miRNAs) and circular RNAs, in the cardioprotective effects of AS-IV. miRNAs, which regulate gene expression post-transcriptionally, have garnered significant attention. Notably, miR-1 has emerged as an upregulated, muscle-specific miRNA in rats following myocardial infarction (MI) [88]. Wang *et al.* (2022) demonstrated that AS-IV (80 mg/kg) mitigated LPS (10 mg/kg)-induced cardiac dysfunction in SD rats by inhibiting miR-1-mediated inflammation and autophagy. This mechanism was recapitulated in vitro, where AS-IV (10 μg/mL) alleviated LPS-induced damage in H9C2 cells through the same pathway [89].

As the field of non-coding RNA research continues to evolve, it becomes increasingly evident that

the cardioprotective effects of AS-IV may be partially mediated by miRNAs and circular RNAs. A more comprehensive and nuanced understanding of their diagnostic potential and regulatory functions within the cardiac system is gradually unfolding, offering fresh insights that could inform the expansion of AS-IV's clinical applications in cardiovascular diseases. This advancement holds promise for improving the prognosis of cardiovascular disease patients, thereby alleviating the financial and emotional burdens on society.

Hence, it is crucial to acknowledge that the multifaceted therapeutic effect of AS-IV, while promising, may also encompass potential side effects that could be inadvertently overlooked by researchers or remain undetected within the confines of shorter experimental timelines. Consequently, unraveling the direct targets of AS-IV's action is imperative. Presently, the precise direct target of AS-IV remains elusive. The exploration of direct targets for active components in traditional Chinese medicine (TCM) represents a pivotal research frontier that merits heightened attention in the future. To address this complexity, modern methodologies and technologies, including systems biology, network pharmacology, molecular docking, and drug target databases, can be harnessed to facilitate preliminary predictions of drug targets. Subsequently, these predictions can be rigorously validated through advanced techniques like surface plasmon resonance (SPR), isothermal titration calorimetry, and cellular thermal shift assay (CETSA), ensuring a robust understanding of AS-IV's mechanism of action. Moreover, it is paramount to recognize that the aforementioned research endeavors have yet to be substantiated in clinical settings. Therefore, future endeavors must persist in the pursuit of novel drug development and a deeper comprehension of AS-IV's mechanism of action, endeavors that have the potential to revolutionize therapeutic strategies for cardiovascular diseases, offering fresh perspectives and innovative solutions.

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Disclosure statement

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