



Review Article

A review of extraction, pharmacological effects, pharmacokinetics and clinical applications of Astragaloside IV

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Abstract: Astragaloside IV (AS-IV), is the primary active compound found in *Astragalus membranaceus*, a functional food for tonifying Qi. Modern extraction technologies, such as ultrasonic extraction, microwave extraction, and enzymatic-assisted extraction, have been utilized to extract AS-IV from *Astragalus membranaceus*, which could improve the extraction efficiency of AS-IV. Extensive scientific researches have revealed that AS-IV has a broad spectrum of pharmacological benefits, including anti-inflammatory, anti-fibrotic, anti-oxidative stress, anti-diabetic, and cardioprotective properties. The multiple effects are achieved through regulating the diverse cellular signaling pathways (MAPK, HIF-1 α and NF- κ B, etc.), which provides a comprehensive strategy for treating various diseases. Due to the multiple effects of AS-IV, this compound has essential therapeutic effects in treating heart failure, myocarditis, cardiac fibrosis and ischemic stroke. Nevertheless, AS-IV possessed short half-life and low bioavailability, which limited the clinical application. Drug delivery systems should be conducted to enhance the absorption rate and AS-IV. Given these diverse and promising effects, AS-IV stands out as a promising candidate for the development of novel therapeutic agents.

Keywords: astragaloside IV; pharmacological effects; pharmacokinetics; clinical applications

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1 Introduction

Astragalus membranaceus, a commonly used Chinese herb, is a plant of the Fabaceae family. *Astragalus membranaceus*, firstly recorded in "Shen Nong's Herbal Classic", is listed as a "top-grade" herb^[1]. The herb is known for its effects of replenishing Qi, securing the exterior, promoting diuresis to reduce edema, and healing sores to promote tissue regeneration^[2]. Moreover, *Astragalus membranaceus* has been proven to possess obvious immune-regulation effects. Nowadays, *Astragalus membranaceus* has also been used as function food, and some products have been developed, such as skin care products and functional beverages. The 2020 edition of "Chinese Pharmacopoeia" contained its related traditional Chinese medicine preparations, such as Huangqi Shengmai Keli, Huangqi Jianwei Gao, and Huangqi Keli^[3]. Modern pharmacological researches have indicated that *Astragalus membranaceus* had anti-inflammatory^[4], anti-tumour^[5], neuroprotective^[6], anti-diabetic^[7], anti-oxidative^[8] and immunomodulatory effects^[9].

So far, more than 100 compounds have been identified in *Astragalus membranaceus*, including flavonoids, saponins, and polysaccharides^[10]. Astragaloside IV (AS-IV) is a monomer

compound isolated from total saponins of *Astragalus membranaceus* with multiple activities. It is an important marker component for evaluating the quality of *Astragalus membranaceus* and the related preparations. It has been reported that AS-IV can effectively treat various diseases, such as cerebral ischemia/reperfusion injury, cardiovascular diseases, lung diseases, cirrhosis, and diabetic nephropathy (DN). Moreover, increasing evidence indicated that the treatment effects of AS-IV were closely associated with its anti-fibrosis, anti-inflammatory, anti-oxidative stress, and anti-apoptosis activities. However, few reports on AS-IV for the treatment of cirrhosis and DN were reviewed.

This paper systematically reviewed the extraction, pharmacological effects and pharmacokinetics, aiming to provide valuable information for future research and clinical application of AS-IV related drugs.

2 Chemical constituents of AS-IV

AS-IV is a triterpenoid saponin with the molecular formula $C_{41}H_{68}O_{14}$ and a molecular weight of 784.97 g/mol. The chemical structure of AS-IV can be divided into three parts: A hydroxyl cyclopentane saturated tricyclic parent nucleus, a sugar moiety

connected to the parent nucleus, and a polyhydroxy chain. Specifically, the parent nucleus of AS-IV is cyclopentane diterpene acid, and the sugar moiety is composed of one α -L-rhamnose and two β -D-glucoses (Fig. 1). AS-IV possessed diverse biological activities due to its complex chemical structure. Through the binding free energy calculations by MM-GBSA protocol and the pharmacophore modeling method, researchers found that the highly hydrophobic surfaces in the hydrophobic sites are the most favorable binding locations of AS-IV, and the hydrophobic interactions play a very essential role to exert the multiple effects^[11].

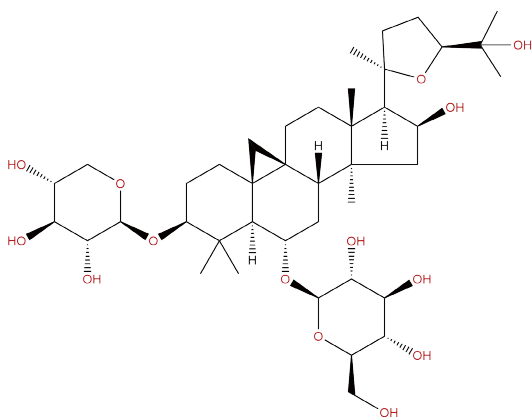


Figure 1 The chemical structure of AS-IV.

3 Extraction of AS-IV

The chemical compounds of *Astragalus membranaceus* are quite complex, and the content of AS-IV is relatively low, ranging from 0.095% to 0.223%. Its low content brought great challenges to the extraction and separation of AS-IV. Traditional extraction methods of AS-IV from *Astragalus membranaceus* included hot reflux extraction and solvent extraction. Although these traditional extraction methods have low equipment costs, they have obvious disadvantages of long-time consumption, low efficiency and hard recovery. Modern extraction approaches significantly increased the extraction efficiency and recovery rate of AS-IV. Ultrasonic extraction and microwave assisted extraction technologies utilize the ultrasound and microwave to destroy the plants cell walls, thus achieving high extraction of AS-IV (more than 60%). Other modern extraction methods, such as enzymatic-assisted extraction, supercritical fluid extraction and flash evaporation extraction, have also been used to extract AS-IV, which could reduce the consumption of organic solvents and increase the

extraction recovery of AS-IV. Recently, high-speed counter-current chromatography has been applied for the separation and purification of AS-IV, and large amounts of high-purity AS-IV could be prepared^[12]. Common extraction methods are listed in the Table 1.

4 Pharmacological properties of AS-IV

4.1 Anti-inflammatory effects and antioxidant effects

Inflammation is a defensive response of the body to various damaging factors such as trauma and infection. The purpose of inflammation is to maintain the internal environment stability and health of the body through a series of defense reactions, such as eliminating pathogens, removing damaged tissues and cells, limiting the spread of pathogens, and initiating the repair process. However, excessive inflammation can cause inflammatory damage to the body. AS-IV is considered to have significant anti-inflammatory effects. AS-IV regulated the occurrence of inflammatory responses through various pathways, such as inhibiting the production and release of pro-inflammatory factors (IL-6, TNF- α , and IL-1 β , etc.), regulating immune cell activity, and inhibiting the NF- κ B signaling pathway to reduce inflammatory damage. The anti-inflammatory mechanism of AS-IV is shown in Fig. 2. Studies have shown that the NLRP3 inflammasome and NF- κ B could be regulated by calcium sensing receptor (CaSR)^[19], AS-IV could inhibit the activation of NLRP3 inflammasomes induced by high glucose through inhibiting the TLR4/NF- κ B signaling pathway and CaSR^[20]. Atherosclerosis was closely related to inflammatory reactions, an *in vitro* atherosclerosis model indicated that AS-IV protected HUVEC cells from ox-LDL-induced damage by reducing the mRNA expression of TNF- α and IL-6, as well as inhibiting the production of inflammatory cytokines^[21]. Additionally, studies have shown that AS-IV regulated the miR-21/PTEN/PI3K/AKT signalling pathway in adipocytes, thereby reducing insulin resistance and inflammatory responses in adipocytes^[22]. Overall, AS-IV exerted the obvious anti-inflammatory effects through regulating NF- κ B and NLRP3 signaling pathways, so as to treat inflammatory related diseases (myocarditis, diabetes and hyperlipidemia, etc.).

Oxidative stress refers to the imbalance between the production of free radicals and reactive metabolites (oxidants or reactive oxygen species (ROS)) and their elimination through protective mechanisms (antioxidants)^[23]. The excessive production of ROS, could lead to various diseases, including cardiovascular diseases^[24], atherosclerosis, inflammation, cancer^[25], diabetes^[26], osteoporosis^[27], and Alzheimer's disease^[28]. The antioxidant

Table 1 The extraction methods of AS-IV

Extraction method	Extraction principle	Advantages	Disadvantages
Solvent extraction ^[13]	Utilizing the solubility difference of target component in a specific solvent for extraction	Simple operation, low equipment requirements	Environmental pollution and low extraction efficiency
Ultrasonic extraction technology ^[14]	Utilizing the ultrasound in a solvent to destroy the cell wall and improve extraction efficiency	Fast extraction speed, simple operation, high extraction efficiency	High equipment cost, small scale, long-term operation, poor stability
Microwave-assisted extraction ^[15]	Utilizing microwave energy to selectively heat the target substance, improve its interaction with the solvent	Fast extraction speed, high efficiency, selective heating	High cost of equipment, large fluctuation in purity, limited for large-scale production
Enzymatic-assisted extraction ^[16]	Utilizing enzymes to degrade the cell wall and release the target component	Highly specific, high extraction efficiency, mild conditions	High cost of enzymes, possible enzyme residue problems
Supercritical fluid extraction ^[17]	Utilizing the special physical properties of supercritical fluids for the extraction of target components	No solvent residue, high purity of extract, environmentally friendly	Large equipment investment, high operating cost, low selectivity for some substances
Flash evaporation extraction ^[18]	Using high-speed shear force and kinetic molecular filtration technology for extraction	Fast extraction speed, high efficiency, and operated at room temperature	High equipment cost, limited for large-scale applications

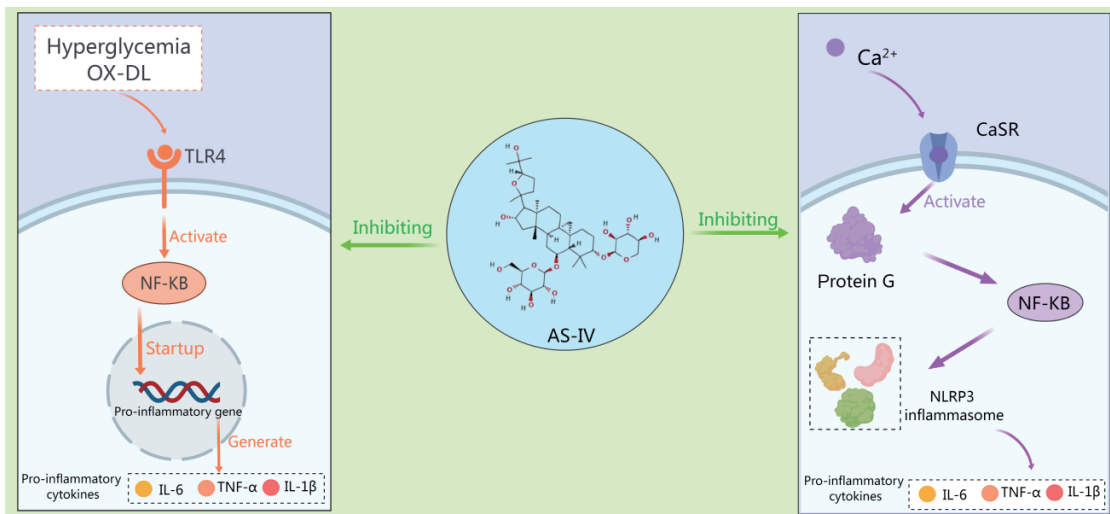


Figure 2 The anti-inflammation effects of AS-IV. AS-IV inhibited high glucose-induced activation of the NLRP3 inflammasome by suppressing the TLR4/NF-κB signaling pathway and CaSR.

mechanism of AS-IV was shown in Fig. 3. By inhibiting the ROS/Caspase-1/GSDMD signaling pathway, AS-IV mitigated myocardial fibrosis and cardiac remodeling induced by myocardial infarction (MI)^[29]. Moreover, AS-IV may alleviate cognitive impairment in type 2 diabetes mellitus (T2DM) mice by reducing oxidative stress and neuroinflammation through regulating the Nrf2/Keap1/HO1/NQO1 signaling pathway^[30]. AS-IV played essential roles in the DN, it could inhibit palmitate-induced oxidative stress and fibrosis in human glomerular mesangial cells (HMC) by downregulating CD36 expression, mediating free fatty acid (FFA) uptake and lipid accumulation^[31]. In summary, AS-IV exerted the antioxidant activities *via* regulating Nrf2/ARE and ROS/Caspase-1/GSDMD signaling pathways.

4.2 Immunomodulatory effects

AS-IV possessed significant effects in regulating immune function. AS-IV can increase the proliferation of T cells and B lymphocytes, as well as the production of antibodies. *In vitro* experiments also showed that AS-IV can affect inflammation by inhibiting the pro-inflammatory factor high mobility group box 1 (HMGB1). HMGB1 has a potential effect on regulatory T cells (Treg), indicating the role of AS-IV in regulating immunity^[32]. Furthermore, AS-IV significantly improved T immune imbalance through regulating Th17/Treg cells in rat depression model. Studies have shown that AS-IV can be used as a promising dietary or dietary combination to alleviate depressive symptoms^[33]. AS-IV, as a food additive, can enhance the antioxidant capacity

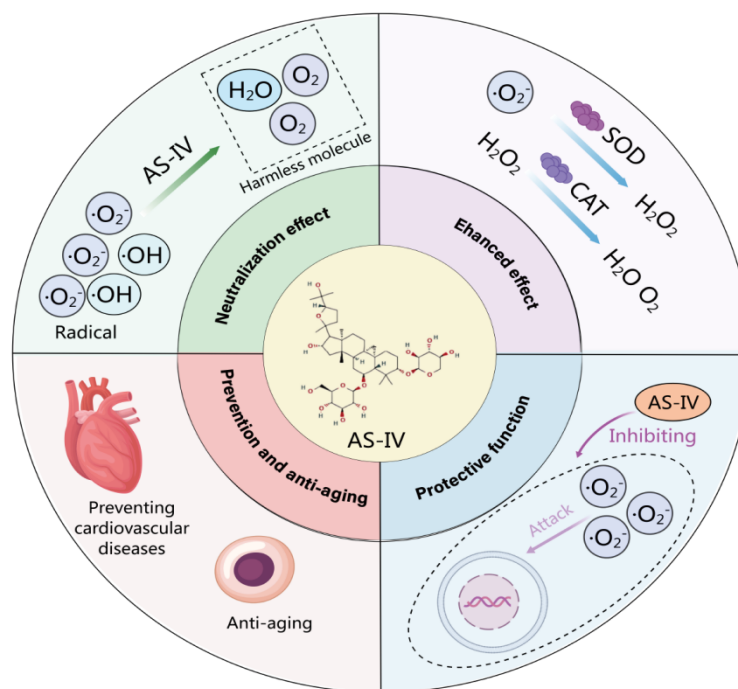


Figure 3 The anti-oxidant effects of AS-IV. AS-IV alleviated oxidative stress through various pathways, such as directly scavenging free radicals (superoxide anion, hydroxyl radical, etc.), upregulating the expression of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), etc.), inducing the expression of antioxidant genes, and inhibiting the activity of oxidases, thereby protecting cells from oxidative damage.

and immune function of tilapia, which may be related to the ability of AS-IV to scavenge free radicals, reduce lipid peroxidation levels, and promote the secretion of immune cells^[34].

4.3 Anti-tumor effects

Recent studies have shown that AS-IV possessed significant anti-tumor activity and had the potential to become an effective therapeutic agent in cancer treatment^[35,36]. The anti-tumor effects of AS-IV primarily included inhibiting the growth and proliferation of cancer cells^[37], promoting cancer cell apoptosis^[38], inhibiting cancer cell invasion and migration^[39,40], blocking the cell cycle progression, inhibiting cancer epithelial-mesenchymal transition (EMT)^[41], enhancing sensitivity to anti-tumor drugs^[42], and enhancing cellular immunity. Multiple studies have demonstrated that AS-IV can regulate macrophage polarization through various pathways to inhibit the development of cancer cells. For example, by inactivating the TGF- β /Smad2/3 signaling pathway, AS-IV can inhibit the polarization of M2 macrophages and subsequently prevent the migration and EMT of cervical cancer cells^[43]. Through regulating the TLR4/NF- κ B/STAT3 signaling pathway, AS-IV can inhibit the proliferation, migration, and invasion of hepatocellular carcinoma cells^[44]. By acting on AMPK signaling, AS-IV can inhibit the progression and metastasis of lung cancer^[45].

4.4 Anti-fibrotic effects

Fibrosis is a pathological repair process involving the replacement of normal tissue with excessive collagen and other extracellular matrix (ECM) components. Fibrosis can affect multiple organs, such as the liver (cirrhosis), lungs (pulmonary fibrosis), heart (myocardial fibrosis), and kidneys (renal fibrosis). The anti-fibrotic mechanism of AS-IV is shown in Fig. 4. Studies have shown that AS-IV delayed the onset of primary hepatocellular carcinoma by continuously inhibiting the development of fibrosis. The therapeutic mechanism involved the regulation of the COOH terminal (pSmad3C), linker region (pSmad3L) of Smad3, and the Nrf2/HO-1 pathway, and Nrf2 phosphorylation^[46]. In the lungs, AS-IV reduced cell pyroptosis and fibrosis development during pulmonary arterial hypertension (PAH) by downregulating hypoxia-inducible factor 1 α (HIF1 α) and increasing the expression of prolyl-4-hydroxylase 2 (PHD2)^[47]. In the heart, AS-IV may prevent atrial fibrosis and atrial fibrillation (AF) induced by angiotensin II (Ang II) by upregulating the SIRT1/PGC-1 α /FNDC5 pathway^[48]. In the

kidneys, AS-IV inhibited palmitate-induced oxidative stress and fibrosis in human glomerular mesangial cells (HMCs) by downregulating CD36 expression, mediating FFA uptake and lipid accumulation^[31]. Furthermore, AS-IV can alleviate renal fibrosis by inhibiting renal tubular epithelial cell pyroptosis induced by urotensin II through regulating the cAMP/PKA signaling pathway^[49].

4.5 Hypoglycemic and Hypolipidemic Effects

Diabetes mellitus (DM) is a common chronic metabolic disorder characterized by insufficient insulin secretion or inadequate response of the body to insulin, leading to persistently elevated blood glucose levels. Several studies have demonstrated that AS-IV had hypoglycemic and hypolipidemic effects, and could be used in the treatment of DM. AS-IV significantly reduced the blood glucose, triglycerides, and insulin levels of diabetic mice, and inhibited the mRNA and protein expression and enzyme activities of liver glycogen phosphorylase and glucose-6-phosphatase^[50]. Similar studies have also indicated that AS-IV can effectively reduce blood sugar levels^[51,52]. In a diabetic rat model, the levels of serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol levels in the middle and high-dose groups of AS-IV were significantly lower than those of the model group ($P < 0.05$), indicating that AS-IV has significant hypolipidemic effects^[53]. Additionally, studies have shown that AS-IV decreased serum triglyceride and total cholesterol levels as well as liver triglyceride contents^[54]. Therefore, from the perspective of lowering blood sugar and lipid levels alone, AS-IV can be considered an ideal choice for the treatment of diabetes.

4.6 Antiviral effects

AS-IV also exhibited significant antiviral activity. Coxsackievirus B3 (CVB3) is a major pathogen leading to viral myocarditis and dilated cardiomyopathy in children and young people. Recent research suggested that AS-IV protected against CVB3-induced myocardial damage and fibrosis, which may partly attribute to suppress the activation of FAS/FASL signaling pathway^[55]. Additionally, it has been reported that AS-IV possessed potent antiviral activity against hepatitis B virus^[56]. AS-IV weakened the innate immune function decline caused by porcine reproductive and respiratory syndrome virus (PRRSV) infection, restored the suppressed cGAS-STING signaling pathway, increased interferon expression, and ultimately exerted antiviral effects^[57]. AS-IV may become a promising candidate for a

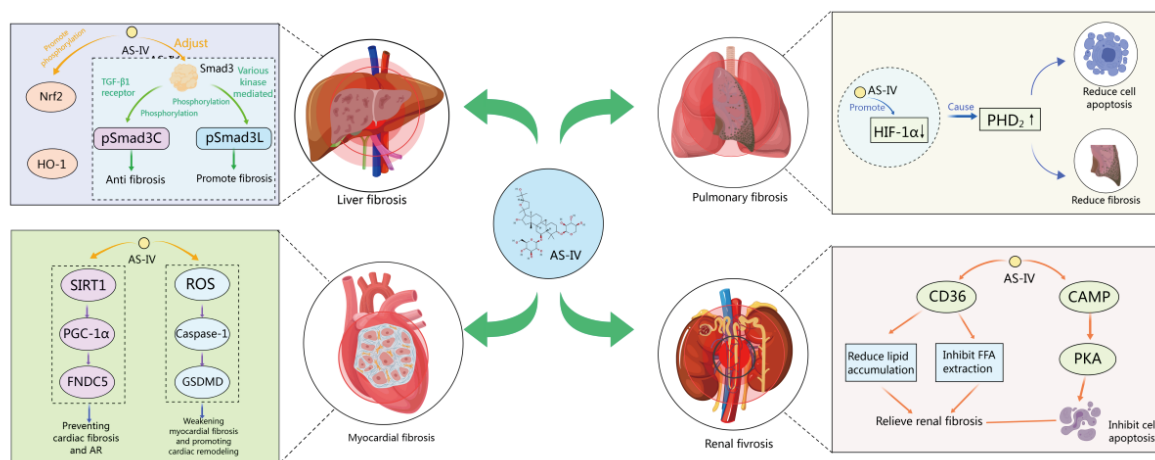


Figure 4 The anti-fibrotic mechanism of AS-IV. AS-IV delayed the onset of primary hepatocellular carcinoma by continuously inhibiting the development of fibrosis.

new antiviral drug against PRRSV. Researchers also found that AS-IV could inhibit the production of reactive oxygen species induced by the influenza virus, suppress the nucleotide-binding oligomerization domain-like receptor pyrin domain-containing protein 3/caspase-1 signaling pathway induced by the influenza virus, and alleviated severe pneumonia induced by Poly(I:C) infection^[58]. Additionally, AS-IV is expected to be a potential drug for alleviating hyperinflammation in COVID-19 by inhibiting the Influenza A pathway and NOD-like receptor signaling pathway^[59]. The overlapping targets of AS-IV related targets and SARS-CoV-2 DEGs can be considered as potential drug targets in the treatment of COVID-19. Therefore, we believe that AS-IV is likely to be beneficial in treating severe COVID-19 patients.

5 Pharmacokinetics properties of AS-IV

Studies have shown that AS-IV has a relatively low absorption and bioavailability in the gastrointestinal tract. In rat models, the oral bioavailability of AS-IV is approximately 2.2% to 3%; while in dog models, it is slightly higher, reaching 7.4%^[60-63]. The low absorption rate may be related to its large molecular structure and low lipophilicity. Interestingly, in liver metabolism studies, the metabolism of AS-IV is very low, indicating that it undergoes minimal first-pass metabolism before entering the systemic circulation. AS-IV's low metabolic rate means that it can enter the bloodstream at higher concentrations, potentially leading to greater pharmacological effects.

When AS-IV is administered intravenously at a dose of 4 mg/kg, it is rapidly absorbed and distributed widely throughout the tissues. In all tissues, the concentration of AS-IV is highest in the liver and kidneys, which is consistent with its pharmacological targets. Additionally, AS-IV also has relatively high concentrations in the lungs, heart, and spleen^[64]. However, the poor BBB permeability of AS-IV limits its accumulation in the brain, which may reduce its therapeutic effects in neurological diseases. Furthermore, it has been found that over 83% of AS-IV was bonded to plasma proteins^[65], which may affect its free concentration and pharmacological effects. Approximately 50% of AS-IV is eventually excreted through urine and feces^[65], indicating that its clearance and excretion mechanisms are relatively clear.

In summary, the pharmacokinetic properties of AS-IV are of significant importance for its clinical application. Future research can explore the pharmacokinetic behavior of AS-IV in different disease states and how to improve its bioavailability and therapeutic effects through optimizing dosing regimens and formulation technologies.

6 Clinical applications of AS-IV

The pharmacological effects of AS-IV included anti-inflammatory, antioxidant, immunomodulatory, anti-fibrotic, and anti-tumor, which suggested that AS-IV could have unique advantages for the treatment of complicated diseases (ischemic stroke, heart disease and diabetes, etc.). AS-IV has relatively small toxic and side effects, and is considered safe for long-term application. AS-IV has been used for the treatment of cerebral ischemia/reperfusion injury^[66], DN^[67], cancer^[44], Alzheimer's disease^[28], asthma^[68], and intervertebral disc degeneration^[69]. Clinical experiments have demonstrated that AS-IV possessed excellent clinical efficacy for the above complicated diseases.

As a commonly used traditional Chinese medicine, *Astragalus membranaceus* has abundant in resources and was easy to obtain.

Furthermore, the research on the pharmacological effects and mechanisms of AS-IV is relatively in-depth, providing a solid scientific foundation for its clinical applications. Therefore, AS-IV has unique advantages in clinical applications, potentially superior to other herbal components in certain cases. However, in practical application, the oral bioavailability and solubility of AS-IV are not very satisfactory.

7 Summary and future prospects

Astragalus membranaceus, as a traditional Chinese medicinal herb, is not only widely used in the medical field but has also gradually been adopted as a functional food in cuisine, exerting its unique medicinal and dietary benefits. In terms of medication, *Astragalus membranaceus* is often used to treat recurrent infections caused by a weakened immune system; in culinary applications.

AS-IV is a natural compound primarily extracted from *Astragalus membranaceus*. It is used in traditional Chinese medicine to enhance the immune system and promote the body's natural healing process. However, AS-IV is not used as a drug, but rather an active ingredient found in some herbal preparations or supplements. Currently, AS-IV has been developed as food supplements, health care products, and functional beverages. Also, the development of AS-IV related drugs has achieved huge progress. HHQ16, an AS-IV derivative, which ameliorated infarction-induced hypertrophy and heart failure through the degradation of lncRNA4012/9456, providing a novel approach for the treatment of cardiovascular diseases^[70]. This finding highlighted the potential of AS-IV and its derivatives in treating complex pathologies such as heart failure.

The research on AS-IV and its derivatives is expected to expand in several key areas. Firstly, there is a possibility for the development of new AS-IV derivatives that can target a broader range of diseases beyond cardiovascular conditions. Secondly, the in-depth study of the action mechanisms of AS-IV and its derivatives will provide a more scientific and precise understanding of their therapeutic effects. Thirdly, the clinical application scope of AS-IV and its derivatives may further expand, with potential applications in mental diseases, autoimmune diseases, and metabolic diseases. Fourthly, the development of new formulations, such as nanoparticles and sustained-release formulations, will enhance the bioavailability and patient compliance. Lastly, comprehensive safety assessments and toxicology studies will ensure the long-term safety and interactions with other drugs. The standardization and quality control of AS-IV and its derivative drugs will guarantee their quality and efficacy.

However, due to the outdated experimental data on the pharmacokinetics of AS-IV, there are still some gaps in understanding the specific metabolic processes, metabolites, pharmacokinetics, and pharmacodynamics of AS-IV in the body. Moreover, to date, most studies on the biological activity of AS-IV have been conducted in animal and cell models, with a lack of research examining the therapeutic efficacy of the drug in clinical settings. Future efforts are needed not only to validate the clinical potential of AS-IV, but also to expand the knowledge of its toxicity and pharmacokinetics in the human body.

Data availability

All data needed to support the conclusions in the paper are

presented in the manuscript. Additional data related to this paper may be requested from the corresponding author upon request.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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Author contribution statement

Li Shi: Data curation, literature search, writing—original draft, visualization. Tian-Sheng Yu: Data curation, validation, writing—review & editing. Chen-Ling Gu: Writing—review & editing, visualization. Ting-Yu Bao: Data curation, validation. Shi-Rong Sheng: Writing—review & editing, project administration. Yang Zhou: Investigation, writing—review & editing. Yong-Juan Zhao: Supervision, funding acquisition, project administration, writing—review & editing. Han-Qing Pang: Supervision, funding acquisition, project administration, writing—review & editing. All the authors have read and approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

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