

Research Progress on Anti-Apoptosis Mechanism of Astragalus Polysaccharides

Xinchen Wang^{1*}, Yanchun Zhang²

¹ Department of Traditional Chinese Medicine, First Clinical Medical College, Shaanxi University of Chinese Medicine, Xianyang, China

² Shaanxi Eye Hospital, Xi'an People's Hospital (Xi'an Fourth Hospital), Affiliated People's Hospital of Northwest University, Xi'an Ocular Fundus Disease Research Institute, Xi'an, China

Email Address

wangxinchen97@163.com (Xinchen Wang)

*Correspondence: wangxinchen97@163.com

Received: 2 February 2024; **Accepted:** 28 February 2024; **Published:** 13 March 2024

Abstract:

Apoptosis is an active process of self-destruction of biological cells, and it is an important mechanism for multicellular organisms to regulate the development of the body, control cell aging and maintain the stability of the internal and external environment of the body. Astragalus membranaceus, as a traditional Chinese herbal medicine, has a long history of clinical application. Astragalus polysaccharide (APS) is the main active ingredient extracted from the roots of Astragalus membranaceus, which has been widely used in the treatment of diseases, such as respiratory system, digestive system, urinary system, blood system, diabetes and its complications. Modern pharmacological studies have found that APS plays an outstanding role in preventing apoptosis. In this paper, the mechanisms of regulating apoptosis by APS are reviewed.

Keywords:

Cell Apoptosis, Astragalus Polysaccharide, Extrinsic Pathway, Mitochondrial Pathway, Endoplasmic Reticulum Stress Pathway

1. Introduction

Apoptosis is a specific form of programmed cell death which occurs in multicellular organisms under normal physiological conditions to support different biological systems including normal cell turnover, immune system, tissue homeostasis, embryonic and brain development. [1] The intricacy of apoptosis has been a focus of an array of researches. At present, there are three known pathways of apoptosis: the first pathway is mediated by death receptors on cell membrane, such as Fas and tumor necrosis factor receptor family, which is called extrinsic pathway. The second pathway involves the release of cytochrome C and the activation of Caspase-3, an effector enzyme of cysteine protease family, which is called mitochondrial pathway. The third pathway is called endoplasmic reticulum stress pathway, which is mediated by endoplasmic reticulum apoptosis signal or Caspase-12. [2,3] Astragalus membranaceus is one of the most widely used traditional Chinese herbal medicines, it has many pharmacological effects such as strengthening the body resistance,

invigorating the middle energizer and benefiting qi. Astragalus polysaccharide (APS) one of the main natural active components of *Astragalus membranaceus*. Contemporary use of APS mainly focuses on its strong pharmacological effects, such as anti-inflammatory, anti-oxidation, anti-apoptosis, reducing blood sugar and lipid, anti-aging, anti-tumor, enhancing immunity and so on. [4] A large number of scholars' experimental studies show that the protective mechanisms of APS in inhibiting apoptosis mainly involve extrinsic pathway, mitochondrial pathway and endoplasmic reticulum stress pathway. [5] The relevant mechanisms are summarized as follows.

2. Inhibit FAS/FASL Signaling Pathway

Extrinsic pathway is activated by the extracellular death signal, which binds to the corresponding death receptor on the membrane. Passing into cells activates Caspase-2, Caspase-8, Caspase-10 and other cysteine proteases, and then produces a series of downstream biological effects. [6] Cell death receptor belongs to the transmembrane protein of tumor necrosis factor receptor (TNFR) family, among which Fas/FasL is the most representative. [7] Fas protein is a type I transmembrane glycoprotein widely distributed on the cell surface, which is divided into three parts: extracellular region, transmembrane region and intracellular region. FasL protein is a ligand of Fas protein and a type II transmembrane glycoprotein. The mechanism of apoptosis induced by Fas protein begins with the combination of Fas and FasL. Studies have shown that increasing the expression of Fas and FasL can promote apoptosis, while inhibiting the expression of Fas can reduce apoptosis. Recent studies have also shown that Fas/FasL system can induce islet β cell apoptosis and participate in the pathogenesis of diabetes. Immunohistochemical results show that APS intervention can inhibit the expression of Fas in islet β cells, and electron microscope results show that the apoptosis of islet β cells in APS treatment group is significantly reduced. [8,9] Advanced glycation end products (AGEs) in diabetic patients can significantly increase FAS secretion in vascular endothelial cells. APS can reduce the level of FAS induced by AGEs, thus inhibiting the apoptosis of vascular endothelial cells. [10] With the development of percutaneous coronary intervention (PCI), how to avoid and reduce myocardial reperfusion injury (MRI) after PCI has always been the focus of cardiovascular intervention. At present, it is found that Fas/FasL system plays an important role in MRI. Fan et al. demonstrated that APS can inhibit the increase of Fas and FasL genes, and reduce the apoptosis of human cardiac microvascular endothelial cells after ischemia-reperfusion injury by inhibiting Fas/FasL signaling pathway. [11]

3. Protect Mitochondrial Function

3.1. Reduce oxidative stress damage

Reactive oxygen species (ROS), an oxidative stress marker, is mainly produced by mitochondria. A large number of ROS will cause mitochondrial dysfunction and mitochondrial membrane permeability, which will lead to cell apoptosis. Astragalus polysaccharides (APS) have a very good therapeutic effect in the treatment of neurodegenerative diseases and nerve injury disease. Liu et al. explored the effects of APS on the protection of neurons and mitochondria in a mouse model of Parkinson disease (PD) using behavioral experiments, and observations of mitochondrial structure and transmembrane potential. It was shown that APS could reverse MPTP-

induced mitochondrial structural damage, and reduce MPTP-induced high levels of ROS and increase MPTP-induced decrease in mitochondrial membrane potential. In addition, it also decreased the bax/bcl2 ratio, and cytochrome-c and caspase-3 protein content ($P < 0.01$) in substantia nigra in our mouse PD model. [12] Zhang et al. reported that APS up-regulates the proteins Bcl-2, Bcl-xl, and down-regulates the proteins Bax and Bak, which induces a decrease in mitochondrial membrane potential, which induces the release of Cyt-c and AIF, which leads to caspase-dependent and caspase-independent pathway to cause apoptosis. [13] Chang et al. reported that APS can decrease intracellular ROS level, increase activity of SOD, GSH-Px and lower level of MDA and NO in diabetic cardiomyopathy (DCM) cell model, indicating APS exerted antioxidative function in DCM model cells. [14] Diabetic retinopathy is also one of the common complications of diabetes, metabolic memory contributes to the development of diabetic retinopathy. Liu et al. used QPCR to examine the levels of miR-195 and Bcl-2. MDA and SOD detection assays were used to examine the oxidative stress level. APS alleviated the oxidative stress, mitochondrial damage and cell apoptosis induced by high glucose treatments in RPE cells via regulating miR-195. [15] Another report showed that APS reduced the cisplatin-induced mitochondrial and intracellular ROS generation. Furthermore, APS protected the normal morphology of mitochondria, blocked the cisplatin-induced mitochondrial permeability transition pore opening, and reduced the cytochrome leakage. Subsequently, APS reduced the cisplatin-induced apoptosis in mice renal and HK-2 cells. [16]

3.2. Improve energy metabolism

Mitochondria are extremely sensitive to ischemia and hypoxia. When cells suffer from ischemia and hypoxia, ATP content in mitochondria drops sharply. AMPK/PGC-1 α signaling pathway is a newly discovered pathway related to ischemia and hypoxia and energy metabolism regulation. AMPK, as an “energy regulator” and an “intracellular fuel engine”, has attracted much attention. PGC-1 α is a downstream effector molecule of AMPK, which can be directly activated by AMPK and control the dynamic balance of energy by promoting mitochondrial biosynthesis. In the rat model experiment of chronic heart failure, APS increase the ATP content in myocardial tissue, and the number of mitochondria increased gradually, vacuolar degeneration and swelling decreased, mitochondrial crest increased, and myocardial transverse ridges and moistening disc became clear. Besides, the relative expression of PGC-1 α protein, and p-AMPK/AMPK were increased, and reduce apoptosis of cardiomyocytes. [17] Xu demonstrated that APS can increase ATP content, up-regulate the expression of p-AMPK, SIRT1 and PGC-1 α protein, down-regulate SIRT1 mRNA level, and reduce Caspase-9 activity. Finally, maintain the mitochondrial function through AMPK/ SIRT1 /PGC-1 α pathway and protect renal tubular epithelial cells with diabetic nephropathy. [18] In addition, Yu reported that compared with diabetic rat, the expression of Bax, Caspase-3 was falling and the expression of Bcl-2 and PGC-1 α was rising after using APS. The apoptosis index in TUNEL staining was decreased and the HE staining showed that the vacuoles around the nucleus decreased in myocardial tissue. [19] APS increased sperm MMP and ATP content after freeze-thawing, reduced sperm apoptosis by regulating apoptosis-related proteins, and promoted AMPK phosphorylation by activating the AMPK signaling pathway. [20] Irisin is a myokine and adipokine that regulates energy metabolism, and PGC-1 α is the most important factor that regulates the expression of irisin. [21] Guo et al. reported that APS can inhibit the collapse of mitochondrial transmembrane

potential, reduce the opening of permeability transition pore in mitochondrial (mPTP), prevent the destruction of mitochondrial/cytoplasmic barrier and maintain mitochondrial function by regulating PGC-1 α /irisin pathway, thus effectively resisting the apoptosis of myocardial cells caused by H₂O₂. [22]

3.3. Regulate calcium overload

Ca²⁺ can activate a variety of biological enzymes as the second messenger and plays a pivotal role in excitation-contraction coupling. Stable Ca²⁺ concentration inside and outside the cell membrane is the premise of maintaining normal cell function. Mitochondria is important place for Ca²⁺ storage and transportation, and Ca²⁺ in the mitochondria is also involved in the process of oxidative phosphorylation and ATP formation in mitochondria. [23] There are many kinds of Ca²⁺ transport systems on mitochondrial membrane, which are important functional protein complexes for maintaining Ca²⁺ concentration inside and outside cells. When mitochondria are dysfunctional, excessive calcium will be accumulated or absorbed in mitochondria, which will exceed the capacity of mitochondria to form “calcium overload”, which will lead to the decrease of mitochondrial membrane potential and the continuous opening of mPTP, which will cause irreversible damage to mitochondria and cause the death of the whole cell. Astragalus polysaccharide can inhibit the excessive opening of mPTP by reducing Ca²⁺ overload and protecting the membrane potential level, and can inhibit the high expression of Caspase-9 in the upstream, and then inhibit the expression of Caspase-3 in the downstream through the apoptosis cascade reaction. [24]

4. Inhibit Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) stress is a reaction process in which cells activate unfolded protein reaction, endoplasmic reticulum overload reaction and caspase-12-mediated apoptosis pathway in response to misfolding, unfolded protein aggregation and Ca²⁺ disorder in the endoplasmic reticulum cavity. [25] IRE1, ATF6 and PERK are important genes in three signaling pathways of endoplasmic reticulum stress. GRP78 is one of the markers of endoplasmic reticulum stress, and the up-regulation of this gene indicates that endoplasmic reticulum stress increases. HT29 cells have similar biological characteristics to IECs and can be easily obtained by culture. It is an internationally recognized UC inflammatory cell model. [26] Zheng et al. used Thapsigargin to induce HT29 cells, and APS was used to effectively interfere with the PERK/p-eIF2 α signaling pathway so that inhibit the apoptosis of HT29 cells. [27] Another report showed that APS can reduce the relative expression of ATF4, ATF6, GRP78, Bax and caspase3 mRNA in chicken thymocytes induced by Zearalenone, thus alleviating ER stress and apoptosis. [28] APS has been long used as a traditional Chinese medicine in treating diabetes. ER stress-induced apoptosis played a crucial role in the progression of DCM. Sun et al.'s study demonstrated that APS significantly inhibit high glucose stimulated H9C2 cell apoptosis and the expressions of ATF6 and PERK related proteins of ER stress pathway. [29] In the progression of diabetic retinopathy (DR), RPE cells are crucial targets for DR initiation following hyperglycaemia. Peng et al. concluded that APS inhibited ER stress and subsequent apoptosis via regulating miR-204/SIRT1 axis in metabolic memory model of RPE cells. [30]

5. Conclusions

Astragalus membranaceus is a time-honored traditional Chinese medicinal herb in China, with precise therapeutic efficacy, as well as abundant sources, low price and few side effects, and is being widely used in clinical practice. As the main active component of *Astragalus*, APS is composed of glucose, fructose, hexanedioic acid, rhamnose, arabinose, galacturonic acid, etc. It exerts its anti-apoptotic effect mainly through the extrinsic pathway, the mitochondrial pathway and the ER stress pathway. In addition, it has been shown that APS also exerts anti-apoptotic effects by reducing the levels of inflammatory cytokines such as TNF- α , IL-6, IL-1 β , etc. Thereby reducing the inflammatory response. [31] The primary role of autophagy is to clear up damaged cellular components, thus participating in the conservation of different cells. The study of the mechanism of cell autophagy and apoptosis has also received increasing attention. Based on the multi-pathway, multi-target, multi-link action characteristics of traditional Chinese medicine, some scholars have also begun to explore the use of APS to regulate autophagy and thus inhibit the occurrence of apoptosis. [32] In recent years, APS have also been developed into more novel pharmaceutical dosage forms, such as APS nanomedicine (APS-CS/TPP), which in turn have demonstrated more prominent anti-apoptotic ability. [33]

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

- [1] Kari, S.; Subramanian, K.; Altomonte, I.A, et al. Programmed cell death detection methods: a systematic review and a categorical comparison. *Apoptosis*, 2022, 27(7-8), 482-508.
- [2] Gupta, S.; Gollapudi, S. Susceptibility of naïve and subsets of memory T cells to apoptosis via multiple signaling pathways. *Autoimmun Rev*. 2007, 6(7), 476-81.
- [3] Fu, X.; Cui, J.; Meng, X, et al. Endoplasmic reticulum stress, cell death and tumor: Association between endoplasmic reticulum stress and the apoptosis pathway in tumors (Review). *Oncol Rep*. 2021, 45(3), 801-808.
- [4] Zheng Y.; Ren W.; Zhang L, et al. A Review of the Pharmacological Action of *Astragalus Polysaccharide*. *Front Pharmacol*. 2020, 11, 349.
- [5] Lu, L.; Wang, DT.; Shi, Y.; et al. *Astragalus polysaccharide* improves muscle atrophy from dexamethasone- and peroxide-induced injury in vitro. *Int J Biol Macromol*. 2013, 61, 7-16.
- [6] Mughal, M.J.; Xi, P.; Yi, Z, et al. Aflatoxin B1 invokes apoptosis via death receptor pathway in hepatocytes. *Oncotarget*. 2017, 8(5), 8239-8249.

- [7] Liu, G.; Yuan, Y.; Long, M.; et al. Beclin-1-mediated Autophagy Protects Against Cadmium-activated Apoptosis via the Fas/FasL Pathway in Primary Rat Proximal Tubular Cell Culture. *Sci Rep*. 2017, 20, 7(1), 977.
- [8] Li, C.D.; Li, J.J.; Wang, L.; et al. Inhibitory effect of Astragalus polysaccharides on apoptosis of pancreatic beta-cells mediated by Fas in diabetes mellitus rats. *Journal of Chinese Medicinal Materials*. 2011, 34(10), 1579-82.
- [9] Mao, S.M.; Li, C.D.; Wang, L.; et al. Effects of Astragalus Polysaccharide on Ultrastructure and Fas Expression of Pancreatic β Cells in Diabetic Rats. *Chinese Pharmacological Bulletin*. 2010, 26(06), 791-793.
- [10] Zhong, Y.; Chen, C.H.F.; Huang, H, et al. Astragalus Polysaccharides Protects Advanced Glycation End-Products Induced Endothelial Cells Apoptosis. *Clinical Medicine & Engineering*. 2014, 21(01), 18-20.
- [11] Fan, Z.J.; Tang, J.; Xie, L.D.; et al. Study on the expression of Fas/FasL in human cardiac microvascular endothelial cells of myocardial ischemia-reperfusion injury and the effects of astragalus polysaccharides. *China Journal of Traditional Chinese Medicine and Pharmacy*, 2016, 31(11), 4672-4674.
- [12] Liu, H.; Chen, S.; Guo, C.; T et al. Astragalus Polysaccharide Protects Neurons and Stabilizes Mitochondrial in a Mouse Model of Parkinson Disease. *Med Sci Monit*. 2018, 24, 5192-5199.
- [13] Zhang YM.; Zhang LY.; Zhou H.; et al. Astragalus polysaccharide inhibits radiation-induced bystander effects by regulating apoptosis in Bone Mesenchymal Stem Cells (BMSCs). *Cell Cycle*. 2020, 19(22), 3195-3207.
- [14] Chang, X.; Lu, K.; Wang, L, et al. Astraglaus polysaccharide protects diabetic cardiomyopathy by activating NRG1/ErbB pathway. *Biosci Trends*. 2018, 12(2), 149-156.
- [15] Liu, P.; Peng, Q.H.; Tong, P, et al. Astragalus polysaccharides suppresses high glucose-induced metabolic memory in retinal pigment epithelial cells through inhibiting mitochondrial dysfunction-induced apoptosis by regulating miR-195. *Mol Med*. 2019, 25(1), 21.
- [16] Ma, Q.; Xu, Y.; Tang, L.; et al. Astragalus Polysaccharide Attenuates Cisplatin-Induced Acute Kidney Injury by Suppressing Oxidative Damage and Mitochondrial Dysfunction. *Biomed Res Int*. 2020, 2020, 2851349.
- [17] Liu, L.L.; Wang, G.L. Effects of Astragalus Polysaccharides on the Energy Metabolism of Cardiomyocytes in Rats with Chronic Heart Failure. *Chinese Journal of Integrative Medicine on Cardio-Cerebrovascular Disease*. 2022, 20(03), 420-426.
- [18] Xu, X.Y. Effect of astragalus polysaccharide on mitochondrial function of renal tubular epithelial cells with diabetic nephropathy through AMPK /SIRT1 /PGC-1 α pathway. *Shandong Medical Journal*.
- [19] Yu, S.N.; Cao, Q.D.; Lu, M.L, et al. Effect of Astragalus polysaccharide on myocardial cell apoptosis in diabetic rats. *Pharmacology and Clinics of Chinese Materia Medica*. 2015, 31(04), 102-105.
- [20] Zhang, X.; Hu, Z.T.; Li, Y, et al. Effect of Astragalus polysaccharides on the cryopreservation of goat semen. *Theriogenology*. 2022, 193, 47-57.

- [21] Kim, H.; Wrann, C.D.; Jedrychowski, M, et al. Irisin Mediates Effects on Bone and Fat via α V Integrin Receptors. *Cell*. 2018, 175(7), 1756-1768.e17.
- [22] Guo, C.R.; Gu, Y.F.; Niu, Y.J.; et al. Effects of astragalus polysaccharides on H₂O₂ induced oxidative damage and apoptosis in bovine mammary epithelial cells. *Chinese Journal of Evidence-Based Cardiovascular Medicine*. 2022, 14(09), 1083-1087.
- [23] Ye, J.; Wang, R.; Wang, M.; et al. Hydroxysafflor Yellow A Ameliorates Myocardial Ischemia/Reperfusion Injury by Suppressing Calcium Overload and Apoptosis. *Oxid Med Cell Longev*. 2021, 2021, 6643615.
- [24] Fan, Z.J.; Xie, L.D.; Cui, J.; et al. APS post-treatment Protects Against Myocardial Ischemia /Reperfusion Injury by Inhibiting Mitochondrial Damage-Mediated Apoptosis. *Liaoning Journal of Traditional Chinese Medicine*. 2018, 45(07), 1357-1360.
- [25] Ajoolabady, A.; Kaplowitz, N.; Lebeaupin C.; et al. Endoplasmic reticulum stress in liver diseases. *Hepatology*. 2023, 77(2), 619-639.
- [26] Chi, J.H.; Seo, G.S.; Cheon, J.H.; et al. Isoliquiritigenin inhibits TNF- α -induced release of high-mobility group box 1 through activation of HDAC in human intestinal epithelial HT29 cells. *Eur J Pharmacol*. 2017, 796, 101-109.
- [27] Zheng, L.; Wen, X.L.; Duan, S.L. Protective Mechanism of Astragalus Polysaccharide on Thapsigargin-Induced PERK/eIF2 α Pathway-Mediated Apoptosis of HT29 Cells. *Journal of Emergency in Traditional Chinese Medicine*. 2022, 31(02), 222-224+246.
- [28] Hu, H.; Xu, Z.K.; Zhang, K.Z.; et al. Protective Effect of Astragalus Polysaccharides on Zearalenone-Induced Apoptosis of Chicken Thymocytes. *Heilongjiang Animal Science and Veterinary Medicine*. 2022, 10, 109-114.
- [29] Sun, S.; Yang, S.; An, N.; et al. Astragalus polysaccharides inhibits cardiomyocyte apoptosis during diabetic cardiomyopathy via the endoplasmic reticulum stress pathway. *J Ethnopharmacol*. 2019, 238, 111857.
- [30] Peng, Q.H.; Tong, P.; Gu, L.M, et al. Astragalus polysaccharide attenuates metabolic memory-triggered ER stress and apoptosis via regulation of miR-204/SIRT1 axis in retinal pigment epithelial cells. *Biosci Rep*. 2020, 40(1), BSR20192121.
- [31] Wei, Y.; Qi, M.; Liu, C, et al. Astragalus polysaccharide attenuates bleomycin-induced pulmonary fibrosis by inhibiting TLR4/ NF- κ B signaling pathway and regulating gut microbiota. *Eur J Pharmacol*. 2023, 944, 175594.
- [32] Zhang, S.; Dong, K.; Zeng, X.; et al. Astragalus polysaccharide ameliorates steroid-induced osteonecrosis of the femoral head by regulating miR-200b-3p-mediated Wnt/ β -catenin signaling pathway via inhibiting SP1 expression: Astragalus polysaccharide regulates SONFH via SP1. *BMC Musculoskelet Disord*. 2023, 24(1), 369.
- [33] Xu, X.; Rui, S.; Chen, C.; et al. Protective effects of astragalus polysaccharide nanoparticles on septic cardiac dysfunction through inhibition of TLR4/NF- κ B signaling pathway. *Int J Biol Macromol*. 2020, 153, 977-985.



© 2024 by the author(s); licensee International Technology and Science Publications (ITS), this work for open access publication is under the Creative Commons Attribution International License (CC BY 4.0). (<http://creativecommons.org/licenses/by/4.0/>)