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Review

Isorhamnetin: A review of pharmacological effects

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ABSTRACT

Isorhamnetin is one of the most important active ingredients in the fruits of *Hippophae rhamnoides* L. and the leaves of *Ginkgo biloba* L., which possesses extensive pharmacological activities. At present, there have been numerous investigations on isorhamnetin, which has the effects of cardiovascular and cerebrovascular protection, anti-tumor, anti-inflammatory, anti-oxidation, organ protection, prevention of obesity, etc. The related mechanisms involve the regulation of PI3K/AKT/PKB, NF-κB, MAPK and other signaling pathways as well as the expression of related cytokines and kinases. Isorhamnetin has a high value of development and application.

Abbreviations: NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; PKB/, AKT protein kinase B; MAPK, mitogen-activated protein kinases; HO-1, heme oxygenase; Nrf2, nuclear factor erythroid-2-related factor 2; ARE, antioxidant response element; VSMC, vascular smooth muscle cells; ox-LDL, oxidized low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IP3, inositol 1, 4, 5-trisphosphate; NO, netrogen monoxide; FAS, tumor necrosis factor receptor superfamily, member 6; FASL, FAS ligand; LDH, lactate dehydrogenase; CFb, cardic fibroblast; AngII, angiotensin II; TGF-β, transforming growth factor-β; Smad, mothers against decapentaplegic; Aβ, amyloid β-protein; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; VDC, voltage-dependent calcium channel; ROC, receptor-operated calcium channels; GC, guanylate cyclase; GMP, cyclic guanosine monophosphatec; PGI2, prostacyclin 2; ATP, adenosine-triphosphate; COX, cyclooxygenase; ADP, adenosine diphosphate; PAF, platelet activating factor; RBC, red blood cells; GLUT-4, glucose transporter type 4; JAK, Janus kinase; STAT, signal transducer and activator of transcription; PMOP, postmenopausal osteoporosis; RANK, receptor activator of NF-κB; RANKL, RANK ligand; NFATc1, nuclear factor of activated T-cells 1; TRAP, tartrate acid phosphatase; OPG, osteoprotegerin; OC, osteocalcin; ERK, extracellular regulated protein kinases; P38, protein 38; JNK, c-Jun N-terminal kinase; MEK, mitogen-activated protein kinase kinase; RAS, rat sarcoma; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2 associated X protein; PANC-1, pancreatic cancer cell line; ATM, ataxia-telangiectasia mutation; CHK2, checkpoint kinase 2; AST, aspartate transaminase; ALT, alanine aminotransfease; ALI, acute lung injury; LPS, lipopolysaccharide; IκBa, inhibitor of nuclear factor-κB; PXR, progestational hormone X receptor; IBD, inflammatory bowel disease; TLR4, Toll-like receptors 4; TNF-α, tumor necrosis factor-α; IL, interleukin; iNOS, inducible nitric oxide synthase; SMA, smooth muscle actin; EMT, epithelial mesenchymal transformation; ERS, endoplasmic reticulum stress; PKCε, protein kinase C ε; IFN-γ, interferon-γ; DPPH, 1,1-diphenyl-2- picrylhydrazyl; ABTS, diammonium 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate); RPE, retinal pigment epithelial; HIF-1α, hypoxia inducible factor 1α; GSH, glutathione; MPO, myeloperoxidase; AKI, acute kidney injury; IR, ischemia reperfusion; AMPK, adenosine 5′-monophosphate-activated protein kinase; CAMKK2, recombinant calcium/ calmodulin dependent protein kinase kinase 2; AA, arachidonic acid; HSC, hepatic stellate cells; PAI-1, plasminogen Activator Inhibitor 1; PPAR, peroxisome proliferators-activated receptors; ConA, concanavalin A; AFH, acute fulminant hepatitis; APAP, paracetamol; XO, xanthine oxidase; UA, uric acid; MFS, Miaoyao Fanggan sachet; DCs, dendritic cells; BMDCs, bone marrow-derived dendritic cells; CD, costimulatory molecules; CCR7, chemokine receptor 7; Hlα, alpha hemolysin; *S. aureus*, *Staphylococcus aureus*; PGE2, prostaglandin E2; HETE, Hydroxyeicosatetraenoic acid; 5-HT, 5-hydroxytryptamine; SDTNBI, substructure-drug-target network-based inference; MC1R, melanocortin receptor 1; MITF, microphthalmia associated transcription factor; TYR, tyrosinase; TYRP1, tyrosinase-related protein 1; DCT, dopachrome tautomerase; PPARA, peroxisome proliferative activated receptor alpha; PPARD, peroxisome proliferative activated receptor delta; PPARG, peroxisome proliferative activated receptor gamma; ALOX12, arachidonate 12-lipoxygenase; ALOX15, arachidonate 15-lipoxygenase; CBR1, carbonyl reductase 1; OFI, opuntia ficus-indica; NAFLD, nonalcoholic fatty liver disease; UV, ultraviolet

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However, the investigations on its mechanism of action are limited and lack of detailed scientific validation. The manuscript reviewed the pharmacological effects of isorhamnetin and related mechanisms of action for the development of its medicinal properties further.

1. Introduction

Over the last few years, the use of plant-derived drugs has risen significantly in the therapeutic field. There are about 4000 flavonoids in plants, many of which display medicinal properties [1]. Isorhamnetin, a flavonoid compound, is present in the leaves, flowers and fruits of *Hippophae rhamnoides* L. (Fig. 1), *Ginkgo biloba* L. (Fig. 2) and other plants [2]. The fruits of *Hippophae rhamnoides* have the traditional efficacy of invigorating the spleen and eliminating food, relieving cough and phlegm, promoting blood circulation and removing blood stasis. The leaves of *Ginkgo biloba* possess the traditional efficacy of activating blood circulation and removing blood stasis, dredging collaterals and relieving pain, astringing the lung and relieving asthma, and reducing turbidity and lipid. Isorhamnetin is one of the most important active ingredients in the fruits of *Hippophae rhamnoides* and the leaves of *Ginkgo biloba*. Fig. 3 displays the chemical structure of Isorhamnetin, which has a wide spectrum of pharmacological effects, including cardiovascular protection, anti-inflammation, anti-tumor, anti-oxidation, anti-bacterial and anti-virus [2].

Studies have shown that isorhamnetin has a wide range of pharmacological effects on cardiovascular diseases [3] and a variety of tumors [4], and possesses the potential of preventing neurodegenerative diseases [5] such as Alzheimer's disease. It also has the pharmacodynamics against hyperuricemia [6] and pulmonary fibrosis [7]. The pharmacological effects of isorhamnetin are related to its regulation of NF- κB, PI3K/ AKT, MAPK and other signaling pathways and their downstream factors. The pharmacological action and mechanism of isorhamnetin is currently a major research area.

It has been reported that isorhamnetin is cytotoxic to H9C2 cardiomyocytes [8] and mouse primary hepatocytes [9] and induces DNA damage in HepG2 cells [10]. However, present studies on the toxicology of isorhamnetin are limited and further investigations are warranted.

2. Cardio-cerebrovascular and nerve protection

Cardiovascular and cerebrovascular diseases are on the rise and place significant economic burden on nations throughout the world. Cardiovascular diseases tend to elicit more serious diseases. For example, atherosclerosis leads to myocardial fibrosis and gradually develops into heart failure. Isorhamnetin has a wide range of preventive and therapeutic effects on cardiovascular and cerebrovascular diseases, such as anti-atherosclerosis, protection of endothelial cells, anti-myocardial ischemia, anti-hypotension, anti-hypoglycema, and anti-thrombosis. The protective effects of isorhamnetin on the cardiovascular system are almost related to antioxidation, anti-inflammation and anti-apoptosis properties. In addition, isorhamnetin can also improve nerve function, enhance cognition and memory, and prevent and treat neurodegenerative disorders. Table 1 lists the effects of isorhamnetin on cardiovascular and cerebrovascular and nervous system diseases. The related mechanisms, experimental models and effective dosages are also displayed in Table 1. Isorhamnetin can protect cardiovascular cells against inflammation, oxidative damage and apoptosis by affecting PI3K/AKT and NF-κB signaling pathways. Fig. 4 shows the mechanism of action of isorhamnetin against cardiovascular and cerebrovascular diseases.

2.1. Anti-atherosclerosis

Heme oxygenase (HO-1), an endogenous cytoprotective enzyme, has anti-inflammation, anti-oxidative and anti-apoptotic effects and can protect against atherosclerosis and other diseases [11]. The expression of HO-1 is related to the activation of nuclear factor Nrf2 [12] which binds to the antioxidant response element ARE in nuclei to increase the expression of downstream antioxidant enzyme genes or proteins. The PI3K/AKT pathway is related to Nrf2nuclear transcription [13]. The phosphatidylinositol 3-kinases (PI3Ks) can regulate different cell functions such as differentiation, proliferation, apoptosis, and glucose transport. AKT, also named as protein kinase B (PKB) and the important downstream effector of PI3K, can phosphorylate many downstream factors such as enzymes, kinases and transcription factors to regulate cell function [14]. The effect of isorhamnetin on P13 K/AKT pathway is most probably dose-dependent. Yun et al. [15] reported that the stimulation of oxidized low density lipoprotein raised the level of intracellular ROS free radicals, and isorhamnetin at a concentration of 20 mg/kg activated the P13 K/AKT pathway, increase the expression of Nrf2/HO-1, reduced ROS levels and macrophage apoptosis, and inhibited the formation of atherosclerotic plaque in mice. However, the investigation from Gao et al. [16] showed that the use of 100 mg/kg/day of isorhamnetin inhibited the PI3K/AKT signaling pathway and reduced myocardial hypertrophy and fibrosis caused by pressure load. Therefore, isorhamnetin also has the potential to prevent myocardial hypertrophy, but the specific mechanism needs further validation. Meanwhile, isorhamnetin has protective effects against H_2O_2 [8] and hypoxia/ reoxygenation [17,18] -induced cardiomyocyte injury due to the activation of Nrf2/HO-1 mediated oxidative signaling pathway, this being mainly related to anti-oxidation and anti-apoptosis.

In addition, Chen et al. [19] found that isorhamnetin inhibited vascular smooth muscle cells (VSMC) proliferation and collagen and DNA synthesis in a dose-dependent manner. Phenotypic transformation, subintimal migration, proliferation and collagen synthesis of VSMC are the basic pathological characteristics of atherosclerosis. Isorhamnetin is probably useful for clinical prevention and treatment of atherosclerosis.

2.2. Endothelial protection

Isorhamnetin exerts protective effects on endothelial cell injury caused by H_2O_2 [20,21] and ox-LDL [22] owing to its effects of anti-inflammation, anti-oxidation and anti-mitochondria-dependent apoptosis [23]. Isorhamnetin can inhibit the release of calcium from IP3 sensitive calcium pool to protect human umbilical vein endothelial cells from the damage elicited by H_2O_2 promoting intracellular calcium

Fig. 1. *Hippophae rhamnoides* L.

Fig. 2. *Ginkgo biloba* L.

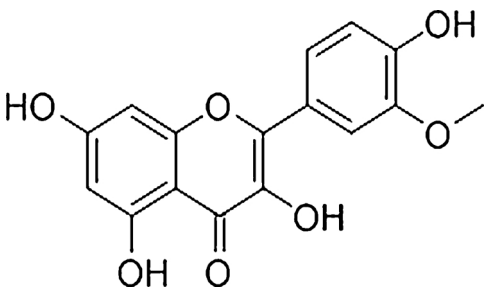

Fig. 3. Chemical structure of isorhamnetin.

release [21]. Caspase-3 possesses an irreplaceable role in apoptosis as the most important terminal cleavage enzyme. NO is an important substance released by vascular endothelial cells and is involved in endothelial dysfunction and apoptosis. The protective effect of isorhamnetin on ox-LDL induced apoptosis in endothelial cells is related to the inhibition of ox-LDL receptor 1 and Caspase-3 mRNA upregulation and the decrease in NO release [22].

Fas and its ligand FasL are membrane surface molecules involved in apoptosis. NF-κB, belonging to transcription factor family, can regulate many molecules related to the early stage of immune response and various stages of inflammatory response. Isorhamnetin can protect cerebrovascular endothelial cells, inhibit FAS/FASL expression and NF-κB translocation, and suppress Fas-mediated apoptosis and cell DNA damage. It may be used in the treatment of cerebrovascular diseases elicited by hyperglycemia and local ischemia [23].

2.3. Anti-myocardial ischemia

Ischemic injury of ventricular myocytes is a common cardiovascular disease and ischemic myocardial reperfusion is widely used to rescue injured tissues. However, it also leads to irreversible myocardial damage. Isorhamnetin can alleviate the damage of Ischemia/reperfusion to ventricular myocytes by inhibition of lactate dehydrogenase (LDH) -elicited apoptosis via NF-κB signaling pathway [24].

2.4. Anti-myocardial fibrosis

Myocardial fibrosis is an important cause of heart failure and involves cardiac fibroblast (CFb) proliferation and extracellular matrix deposition. AngII is a growth regulator of CFb and can combine with the receptor in CFb to promote CFb proliferation and the extracellular matrix synthesis. Isorhamnetin inhibits the proliferation of CFb and collagen synthesis induced by AngII in a concentration-dependent manner, thus preventing myocardial fibrosis [25]. TGF-β is a cytokine that adjusts cell differentiation, proliferation, apoptosis and extracellular matrix synthesis. Smad is involved in intracellular TGF-β signaling and isorhamnetin can alleviate myocardial collagen aggregation and fibrosis in diabetic rats through repressing the TGF-β/Smad signaling pathway [26].

2.5. Anti-cerebral ischemic injury

In the filament model of middle cerebral artery reperfusion in experimental stroke mice, isorhamnetin can reduce the infarct volume, improve nerve function and protect the brain from ischemic injury. Therefore, isorhamnetin could be used for the treatment of ischemic stroke [27]. However, the specific mechanism of action is still unclear and further experimental verification is needed.

2.6. Neuroprotection and improvement of memory and cognition

Isorhamnetin can be used as a potential medicine for the prevention and treatment of various neurodegenerative disorders. Isorhamnetin potentiates the nerve growth factor- induced neurite outgrowth [28]. Amyloid β-protein (Aβ) can be secreted by a variety of cells and has strong neurotoxicity after precipitation and accumulation of cell matrix. Besides causing neurodegenerative diseases, Aβ also leads to the destruction of blood-brain barrier. Isorhamnetin can destroy the stability of Aβ aggregates, protect cells from Aβ-induced cytotoxicity and reverse the cell morphological changes induced by Aβ in human neuroblastoma SH-SY5Y cells. Thus isorhamnetin has a potential to prevent the initiation of Alzheimer's disease (AD) [29].

The open field test and morris water maze test indicate that isorhamnetin can improve the spatial and non-spatial learning and memory impairment induced by scopolamine. These effects are associated with enhanced antioxidant defense systems, cholinergic signaling, and synaptic plasticity by isorhamnetin [5]. It also enhances the antioxidant defense system by increasing the activity of antioxidants in the prefrontal cortex and hippocampus. In addition, isorhamnetin can attenuate scopolamine-induced activity of cholinesterase and brain-derived neurotrophic factor (BDNF) in the prefrontal cortex and hippocampus to enhance cholinergic signaling and synaptic plasticity. Isorhamnetin also decreases the activity of acetylcholinesterase [30]. Therefore, isorhamnetin can be developed as an anti-acetylcholinesterase reagent to prevent neurodegenerative diseases. The enhancing effect of isorhamnetin on cholinergic signal transduction is a primary mechanism for the prevention and treatment of neurodegenerative diseases.

2.7. Hypotensive action

Hypertension is one of the important diseases threatening human health, which could cause serious damage and pathological changes of heart, brain and other organs. Isorhamnetin has the effect of lowering blood pressure and selectivity to resistance vessel and the vasodilation effect is inversely proportional to the diameter of the vessel studied [31]. Hypertensive patients have disorders of cellular calcium regulation, and Li et al. [32] found that isorhamnetin had double inhibitory effects on voltage-dependent calcium channel (VDC) and receptor-operated calcium channels (ROC) of vascular smooth muscle cells (VSMC) in rabbits. It reduced intracellular free calcium level, thereby relaxing blood vessels and lowering blood pressure. Zhu et al. [33] found that isorhamnetin inhibited the increase of intracellular calcium concentration in vascular smooth muscle cells induced by potassium chloride and norepinephrine in spontaneously hypertensive rats and Wistar-Kyoto rats. Isorhamnetin might decrease the levels of Ca^{2+} in VSMCs through blockage of both VDC and ROC in physiological or pathological state, which is probably one of the mechanisms for its hypotensive effects.

Isorhamnetin has endothelium-independent vasodilator effects in aorta, mesenteric arteries and portal vein of rats and coronary arteries of pigs. Isorhamnetin can also induce a positive inotropic effect in

Table 1
Effect of isorhamnetin on cardio-cerebrovascular and nervous system diseases and the involved mechanism.

Therapeutic effect	Experimental subject	Observation	Action	Mechanism of action	References
Anti-atherosclerosis	THP-1; C57BL/6 J mice fed with high fat diet and ApoE-/-mice	In vitro (5, 10, 20 μm), In vivo 20 mg/kg, i.g	Reduce apoptosis of macrophages	Activation of the P13 K/AKT pathway	[15]
Prevention of myocardial hypertrophy	Aortic binding of male C57B/L6 J mice	In vivo 100 mg/kg/day	Decrease angiotensin II induced cardiomyocyte hypertrophy.	Inhibition of PI3K-AKT signal transduction pathway	[16]
Myocardial protection	H9C2 cardiomyocytes stimulated by H_2O_2	In vitro 5, 10, 15, 20 μmol/L	Inhibiting apoptosis	Influence on apoptosis pathway of mitochondria and activation of Nrf2/ARE signal pathway	[8]
Myocardial protection	H9C2 myocardial cell hypoxia/ reoxygenation model	In vitro 3,6,12, 25, 50μM	Inhibiting apoptosis, antioxidation	up-regulation of SIRT1, Nrf2 and HO-1	[18]
Myocardial protection	Hypoxia / reoxidation model of neonatal rat cardiomyocytes	In vitro 10, 20, 40, 60μm	Inhibiting apoptosis	Up-regulation of SIRT1, reduction of cytochrome c release, and reduction of active oxygen generation	[17]
Anti-atherosclerosis	Human vascular smooth muscle cell	In vitro 200, 100, 50, 1 μmol/L	Inhibition of vascular smooth muscle proliferation and collagen synthesis	Undefined	[19]
Endothelial protection	Human umbilical vein endothelial cell line stimulated by H_2O_2	In vitro 22.8, 11.4 and 5.7mug/ ml	Inhibits IP3-sensitive calcium pool release of calcium	Undefined	[21]
Endothelial protection	Human umbilical vein endothelial cell line	In vitro 0.02, 0.2, 2 mmol/L	Inhibiting apoptosis	Interfering with LOX-1-NO- Miton-Caspase-3 Pathway	[22]
Endothelial protection	Human brain microvascular endothelial cells	In vitro 10–100 μmol/L	Inhibition of apoptosis	Inhibition of FAS / FASL expression and NF-κB nuclear translocation	[23]
Anti-myocardial ischemia	Neonatal SD rat ventricular myocyte ischemia/ reperfusion model	In vitro 4μM	Inhibition of apoptosis	Undefined	[24]
Anti-myocardial fibrosis	Rat model of cardiac fibroblasts fibrosis induced by Ang II	In vitro 10^{-4}, 10^{-5}, 10^{-6} mol/L	Inhibition of Cfb proliferation and collagen synthesis	Undefined	[25]
Anti-myocardial fibrosis	Intraperitoneal injection of vermectin 45 mg/ kg in male Wistar rats	In vivo 30 mg/kg, p.o.	Remission of myocardial collagen accumulation and fibrosis in diabetic rats	Inhibition of cardiac TGF-β/Smad signaling pathway	[26]
Anti-cerebral ischemic injury	Experimental cerebral apoplexy model in mice	In vivo 1 mg/mL	Improvement of blood-brain barrier, antioxidation, and anti-inflammation	Undefined	[27]
Neuroprotection-induced differentiation of PC12 cells	PC12 cells	In vitro 1, 3, 10μM	Induction of proliferation	Undefined	
Prevention and treatment of neurodegenerative diseases	SH-SY5Y cells of human neuroblastoma	In vitro 0.1–25 μM	Anti- amyloid β-protein kills SH-SY5Y	Undefined	[29]
Prevention and treatment of neurodegenerative diseases - enhancing learning and memory	Intraabdominal injection of scopolamine (3 mg/kg) in male albino mice	In vivo 1, 5, 50 mg/ kg, p.o.	Enhancement of antioxidant defense system, cholinergic neurotransmission and synaptic plasticity	Increase of glutathione level, superoxide dismutase and catalase activity in the prefrontal cortex and the hippocampus	[5]
Prevention and treatment of neurodegenerative diseases	Microplate analysis of acetylcholinesterase activity	In vitro	Anti-acetylcholinesterase	Binding to the active site of acetylcholinesterase reduces the activity of acetylcholinesterase	[30]
Hypotensive effect	Rabbit aortic vascular smooth muscle cells; Vascular Smooth Muscle Cells in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats	In vitro 10^{-6}～10^{-4}mol/L	Inhibition of voltage-dependent and manipulated calcium channels and reduction of intracellular Ga^{2+} concentration	Undefined	[32,33]

(*continued on next page*)

Table 1 (*continued*)

Therapeutic effect	Experimental subject	Observation	Action	Mechanism of action	References
Hypotensive effect	Isolated thoracic and abdominal aortas of rats	In vitro 3×10^{-6}, 3×10^{-5}, 3×10^{-4}M	Selective diastolic resistance vessel	Undefined	[34]
Hypotensive effect	Isolated rat thoracic aortic rings	In vitro 1～100 μmol/L	Diastolic blood vessel	Activation of NO/GC/cGMP pathway and cyclooxygenase pathway	[35]
Antithrombus	Platelet in healthy volunteers	In vitro 1, 10, 100 μmol/L	Inhibition of platelet aggregation induced by ADP and PAF	Undefined	[37]
Hypoglycemic effect	Rat L6 myoblasts and male ICR mice	In vitro 10^{-2}– 10^{4}nM In vivo 10, 100, 1000 mg/kg	Promotion of glucose uptake in muscle cells	Activation of JAK2/STAT pathway and promotion of GLUT4 translocation	[40]

isolated atria of rats [34]. It has a dose-dependent vasodilative effect on thoracic aortic rings. Low-dose isorhamnetin causes vasodilation through the endothelium-dependent pathway, but this vasodilative effect at high-dose is independent of endothelium pathway. The vasodilative mechanism of isorhamnetin may be related to endothelial NO/GC/cGMP pathway and cyclooxygenase pathway. Promoting endothelial NO production and activating cyclooxygenase increase the production of PGI2, thus exerting vasodilator effect which is irrelevant to ATP-activated potassium channel [35]. Therefore, the hypotensive mechanism of isorhamnetin is mainly endothelium and non-endothelium dependent. At high concentration, non-endothelium-dependent inhibition of calcium channels reduces intracellular calcium levels, but the low concentration promotes endothelial production of NO and activation of COX, thereby relaxing blood vessels and resisting hypertension.

2.8. Anti-thrombus

The formation of thrombus is related to vascular intimal injury, blood properties and blood flow changes. The current primary treatment strategy is to use thrombolytic drugs, antiplatelet drugs, and anticoagulants. Ingesting onion soup rich in isorhamnetin can inhibit collagen-stimulated platelet aggregation and some aspects of signal transduction *in vitro* in a time-dependent manner, reducing the risk of thrombosis [36]. Isorhamnetin can inhibit platelet aggregation caused by either adenosine diphosphate (ADP) or platelet activating factor (PAF) [37]. Therefore, isorhamnetin has the potential to be an antiplatelet medicine for the treatment of thrombus.

2.9. Hypoglycemic effect

Hyperglycemia is also one of the most common diseases in modern society. Sil et al. [38] found that when streptozotocin-induced diabetic rats were administered isorhamnetin-3-O-beta-dextran complex at a dose of 25 mg/kg orally, serum glucose concentration and sorbitol accumulation were significant decreased in the lenses, red blood cells (RBC) and sciatic nerves. Isorhamnetin is indicated for the prevention and treatment of diabetes and its complications. Glucose transporter type 4 (GLUT-4) is a membrane protein that exists only in insulin-sensitive skeletal muscle, myocardium and adipocytes. After stimulation by insulin, GLUT-4 can be transported from stored vesicles to the outer membrane, bind to glucose and transported to cells. It plays an important role in maintaining the body's blood glucose balance. Tyrosine kinase JAK/transcription factor STAT signaling pathway is a signal transduction pathway activated by cytokines, which is relative to cell proliferation, differentiation, apoptosis, and immune regulation. It is also involved in inducing GLUT 4 translocation and maintaining glucose homeostasis [39]. Isorhamnetin activates the JAK2/ STAT pathway and promotes glucose uptake by increasing GLUT4 translocation in different signaling pathways in skeletal muscle cells at a low concentration range, thereby providing beneficial functions for preventing hyperglycemia and maintaining glucose homeostasis [40]. Isorhamnetin might be developed as a hypoglycemic drug for the treatment of hyperglycemia and related diseases.

3. Anti-tumor

Isorhamnetin displays a wide anti-tumor activity, which can inhibit human cervical cancer cells [41,42], lung cancer cells [43–45], colon cancer cells [46,47], breast cancer cells [48–50], pancreatic cancer cells [51], nasopharyngeal cancer cells [52], liver cancer cells [53], gastric cancer cells [54] and other cancer cells. Isorhamnetin inhibits the proliferation of tumor cells, induces apoptosis, and regulates tumor suppressor genes, proto-Oncogenes and signal pathways [4]. Table 2 lists the anti-tumor effects and mechanism of action for isorhamnetin. Fig. 5 shows the anti-tumor mechanism of isorhamnetin.

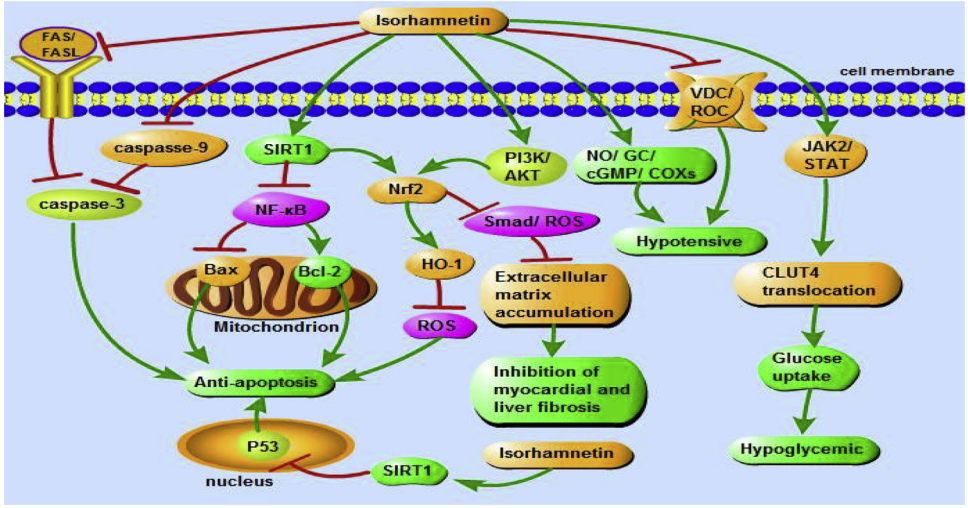

Fig. 4. Mechanism of action of isorhamnetin against cardiovascular and cerebrovascular diseases. According to the figure, it is known that isorhamnetin plays a role in cardiovascular and cerebrovascular diseases by regulating different signal pathways such as PI3K/AKT. (↑: Hints activation or upregulation; ⊥: Hints inhibition or downregulation.).

MAPKs are a class of serine/threonine protein kinases in cells involved in parallel signaling pathways. ERK, P38, JNK and MEK are downstream factors. ERK is the key to the transmission of signals from surface receptors to the nucleus and is involved in many biological reactions such as cell proliferation and differentiation, apoptosis. RAS is its upstream regulatory protein. It has been shown that isorhamnetin can induce cell cycle arrest in G_1 phase, decrease the phosphorylation of cell proliferation pathway proteins AKT and ERK and the expression of proliferating nuclear antigen Ki67, reduce Bcl-2 expression, increase Bax expression, and promote the shear of Caspase 3, inducing apoptosis [50]. Isorhamnetin exerts antitumor effect in breast cancer by regulating Akt and MEK signal transduction pathways [49]. Wang et al. [51] found that isorhamnetin reduced the phosphorylation of MEK and ERK in the Ras/ MAPK pathway, regulated cell proliferation, differentiation and apoptosis in advanced pancreatic cancer cell line PANC-1. Isorhamnetin may be a potential drug to prevent pancreatic cancer. Jiang et al. [53] found that isorhamnetin blocked cells in G_0-G_1 phase, prevented cells from entering S phase of DNA synthesis, and ultimately suppressed the proliferation of HepG-2 cells *in vitro*.

Ataxia-telangiectasia mutation (ATM), a kinase that is activated in the presence of a cellular injury signal, activates the checkpoint effector kinase CHK2 to induce cell cycle arrest [55]. Isorhamnetin can arrest the cell cycle in G_2 / M phase, thus inhibiting cell proliferation of HeLa cells, the mechanism of which is closely related to the activation of ATM-CHK2 pathway and the destruction of microtubule function [42]. Isorhamnetin restrains the proliferation and colony formation of human lung cancer cells (A549) and induces A549 cells apoptosis. The induction of apoptosis may be related to mitochondrial dependent pathway. Through reducing mitochondrial membrane potential, isorhamnetin promotes the release and activation of cytochrome c and caspase and then induces A549 cells apoptosis [45,56]. Isorhamnetin also induces apoptosis by down-regulating carcinogenic genes and upregulating apoptotic genes, hence playing an important anti-tumor role [56].

Isorhamnetin can inhibit the growth of drug-resistant human lung cancer cell lines (PC9-IR) by decreasing the phosphorylation level of Akt473 [43]. Liu et al. [57] found that the antitumor effect of isorhamnetin was also related to the induction of autophagy. In addition, isorhamnetin can inhibit the growth of HeLa cells by inhibiting the activity of telomerase [58].

Cytotoxic effect is an important mechanism for anti-tumor drugs. Dong et al. [8] found that isorhamnetin was cytotoxic to H9C2 cardiomyocytes at the concentration of 80 μmol/L. After incubation of isorhamnetin (30, 100, 300 μmol/L) with rat primary hepatocytes for 24 h, the contents of AST, ALT and LDH in the culture medium were increased, indicating that isorhamnetin possibly caused hepatocyte injury [9]. In addition, Ginkgo biloba leaf extract has been shown to increase the incidence of liver cancer in mice. In order to study the potential carcinogenic mechanism, the human hepatoma cell line HepG2 was used for experiments. It was found that isorhamnetin caused DNA damage and this effect was related to the inhibition of topoisomerase II [10].

In brief, isorhamnetin exerts anti-cancer effects mainly through down-regulating Bcl-2 gene, up-regulating Bax gene, inhibiting telomere activity, decreasing the expression of related proteins to block cell cycle, depressing proliferation and inducing apoptosis.

4. Anti-inflammation

Isorhamnetin has anti-inflammatory effects on many diseases, such as osteoarthritis and periodontitis, which can inhibit inflammatory reactions. In addition, the anti-inflammatory activity of isorhamnetin also plays a role in anti-acute lung injury [59–61], anti-tuberculosis [62] and kidney protection [63,64]. The mechanism is related to regulate the production of inflammatory mediators, cytokines and ROS. Table 3 listed the anti-inflammatory effect and mechanism for isorhamnetin. Some studies have shown that isorhamnetin has a protective effect on LPS-induced acute lung injury model [59,60]. Chi et al. [59] found that isorhamnetin inhibited the phosphorylation of ERK, JNK, IκBa and NF-κB (p65) activated by LPS *in vivo* through affecting the signaling pathways of MAPK and NF-κB and alleviated neutrophil infiltration and edema in ALI model. Isorhamnetin could protect mice from LPS-induced ALI by repressing the expression of COX-2 and suppress LPS-induced inflammation in human gum fibroblasts by activating Nrf2 signaling pathway [65].

The human progestational hormone X receptor (PXR) is a known target for abrogating inflammation in inflammatory bowel disease (IBD) and isorhamnetin is an activator of human progesterone X receptor, which improves the experimental IBD through PXR-mediated up-regulation of xenobiotic metabolism and down-regulation of NF-κB signaling [66]. Kim et al. [67] found that isorhamnetin inhibited LPS-mediated inflammation in BV2 microglia by inactivating NF-κB signaling pathway, antagonizing TLR4 and eliminating ROS accumulation. Isorhamnetin may have potential benefits in inhibiting the onset and treatment of neuroinflammatory diseases. In addition, isorhamnetin has a therapeutic effect on osteoarthritis. It has also shown that isorhamnetin has anti-inflammatory and cartilage protective effects in IL1 β-stimulated cartilage cells [68]. Isorhamnetin inhibits RANKL-induced osteoclast formation and protects chondrocytes from ROS damage by regulating ROS [69]. It exerts anti-inflammatory effects by inhibition of NF-κB signaling pathway and reduction of inflammatory factors release and ROS production [61].

In conclusion, NF-κB pathway plays an extremely important role in

Table 2
Antitumor effect of isorhamnetin and the related mechanism.

Cancer type	Cell type	Observation	Action	Mechanism of action	References
Cervical cancer	Hela	In vitro 20 μg /mL	Inhibition of proliferation and promotion of apoptosis	Induction of G2 / M phase arrest, down-regulation of Bcl-2 gene, up-regulation Bax gene, inhibition of proliferation and induction of apoptosis	[41]
Cervical cancer	Hela	In vitro 1, 10, 100, 1000 μmol/l	Inhibition of proliferation	Induction of G2 / M phase arrest, activation of ATM- Chk2 pathway and destruction of microtubule function	[42]
Lung cancer	A549 cell line and Lew is cell C57BL / 6 mouse transplantation tumor	In vitro 10, 20, 40, 80, 160 μg/m l In vivo Tumor injection 50 mg / (kg·d)	Inhibition of proliferation and induction of apoptosis	Down-regulation of bcl-2 gene and PCNA protein expression, inhibition of DNA synthesis, up-regulation of P53, Bax and caspase-3 genes	[44]
Lung cancer	The gefitinib-resistant PC9(PC9-IR) cells	In vitro 5, 10, 20, 40, 100 μmol/L	Inhibition of tumor growth	Inhibition of PI3K signaling pathway and phosphorylation of Akt473 site, resulting in G2 / M phase arrest	[43]
Lung cancer	A549 cell line	In vitro 0 − 16 μM	Inhibition of proliferation and induction of apoptosis	Activation of Mitochondrial dependent apoptosis pathway	[45]
Colon cancer	HT-29 and Caco2 HCT-116	In vitro	Inhibition of proliferation and induction of apoptosis	Undefined	[46,47]
Breast cancer	MCF7 and BT549	In vitro 3, 10, 30 μmol /L	Inhibition of proliferation and promotion of apoptosis	Induction of cycle arrest in G_1 phase, reduction the phosphorylation of cell proliferation pathway proteins AKT and ERK and the expression of proliferating nuclear antigen Ki67, decrease of the expression of Bcl-2, increase of the expression of Bax, and promotion of the shear of Caspase 3	[50]
Breast cancer	MCF7, T47D, BT474, BT-549, MDA-MB-231, MDA-MB-468	In vitro 0, 10μM	Inhibition of proliferation and promotion of apoptosis	Inhibition of Akt / mTOR and MEK / extracellular signal-regulated kinase phosphate cascade reaction	[49]
Breast cancer	TNBC cells and xenograft mouse	In vitro 2.5, 5, 10, 15μM In vivo Intraabdominal injection of 20 mg/kg	Promotion of apoptosis	Activation of Mitochondrial dependent apoptosis pathway	[48]
Pancreatic cancer	PANC-1 and LoVo	In vitro 10–100 μM	Inhibition of proliferation and migration	Inhibition of Ras / MAPK pathway activity, causing S phase arrest	[51]
Nasopharyngeal cancer	CNE-2	In vitro 10, 20, 40, 80 mg/L	Inhibition of proliferation	Undefined	[52]
Liver cancer	HepG-2	In vitro 20, 40, 60, 80, 100 mg /L	Inhibition of proliferation and induction of apoptosis	Blocking the cells in the $G_0 \sim G_1$ phase	[53]
Gastric cancer	SGC- 7901	In vitro 0.4×10^{-4}, 0.8×10^{-4}, 1.2×10^{-4}, 2.4×10^{-4} mol /L	Inhibition of proliferation	Undefined	[54]
Lung cancer	A549	In vitro 10 ~ 320 μg/ ml	Induction of apoptosis	Up-regulation of the expression of apoptosis genes Bax,Caspase-3 and p53 and down-regulation of the expression of Bcl-2, cyclinD1 and PCNA proteins	[56]
Colon cancer	HCT116	In vitro 0 ~ 100 μmol /L	Inhibitionf of proliferation and induction of autophagy	Undefined	[57]
Cervical cancer	HeLa	In vitro 10, 20, 40, 80 μg /mL	Inhibition of proliferation and induction of apoptosis	Inhibition of telomerase activity	[58]

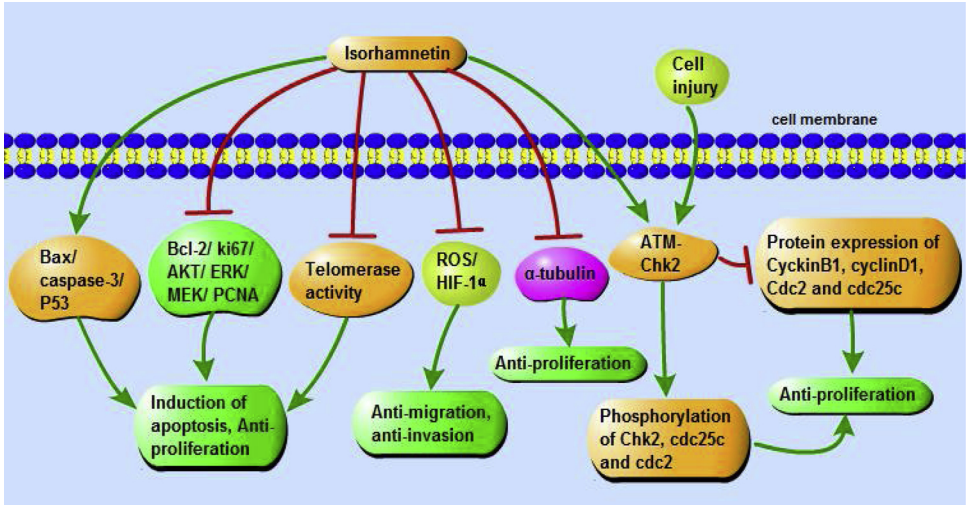

Fig. 5. The anti-tumor mechanism of isorhamnetin. Isorhamnetin inhibits tumor cell proliferation and promotes apoptosis by regulating the expression of tumor-related genes or proteins such as Bcl-2 and Bax.

anti-inflammatory effect of isorhamnetin. Many molecules in all stages of inflammatory response are regulated by NF-κB, including TNF- α, IL-1β, IL-2, IL-6, IL-8, IL-12, iNOS, COX2, chemokines, adhesion molecules, colony stimulating factors plus others. Inhibition of NF-κB pathway is the main anti-inflammatory mechanism of isorhamnetin.

5. Kidney protection

Isorhamnetin has a protective effect on AKI induced IR injury in rats. The mechanism is related to inhibit over-activation of NF-κB signaling pathway, alleviating inflammation and oxidative stress, so it can be used in prevention and treatment of AKI in acute renal injury [63]. Isorhamnetin can ameliorate diabetes-elicited renal damage, which may be relevant to the negative regulation of NF-κB pathway [64]. Isorhamnetin can inhibit the downstream inflammatory factors expression and the inflammatory response through the negative regulation of NF-κB pathway, exerting a renal protective effect. The renal protective effect and mechanism of isorhamnetin are shown in Table 3.

6. Lung protection

6.1. Anti-pulmonary fibrosis

Pulmonary fibrosis is a chronic and progressive disease characterized by alveolar epithelial injury and abnormal collagen production. The pulmonary fibrosis model of C57 mice was established by intraperitoneal injection of a single dose of bleomycin (3.5U/kg), followed by intragastric administration of isorhamnetin. The results showed that isorhamnetin inhibited collagen deposition induced by bleomycin, reduced the expression of type I collagen and alpha SMA, and alleviated epithelial mesenchymal transformation (EMT) and endoplasmic reticulum stress (ERS) *in vivo*. Therefore isorhamnetin can be used to inhibit EMT and pulmonary fibrosis induced by bleomycin [7]. Moreover, isorhamnetin can inhibit Ca^{2+} overload, reduce the production of ROS and inhibit apoptosis through protein kinase C ε (PKCε) pathway to decrease the damage induced by ERS [70]. The anti-pulmonary fibrosis effect and mechanism of isorhamnetin are shown in Table 4.

6.2. Anti-tuberculosis

Tuberculosis is a chronic infectious disease elicited by *Mycobacterium tuberculosis*. Mycobacterium mainly causes strong local inflammation in the lungs, which is essential in the pathogenesis of tuberculosis, and isorhamnetin can inhibit the release of TNF-α and IL-12. Meanwhile, isorhamnetin can decrease the stimulation of IFN-gamma-mediated extracellular signal-regulated kinase and p38 MAPK, and reduce the expression of TNF-α, IL-1β, IL-6, IL-12 and MMP-1 in cells stimulated by IFN-γ, thus inhibiting the inflammatory response [62]. Isorhamnetin can be used as an effective antituberculosis drug due to its anti-inflammatory effect (as shown in Table 3).

7. Anti-osteoporosis

Osteoporosis is a systemic bone metabolic disease marked by low bone mass and bone microstructure destruction, which is one of the serious threats to the elderly and women's health. Isorhamnetin effectively inhibits osteoclastogenesis and bone resorption in rat femoral shaft and tendon tissue and mouse bone marrow cells to prevent osteoporosis [71], but the involved mechanism of action still remains unclear. Chao et al. [72] performed bilateral ovariectomy in rats to construct a model of women postmenopausal osteoporosis (PMOP) for investigating the preventive and therapeutic effects of isorhamnetin on PMOP. RANK ligand (RANKL) was detected in osteoblasts and bone marrow stromal cells. The combination of NF-κB (RANK) receptor activator with RANKL could promote the differentiation and activation of osteoclasts. Osteoblasts and bone marrow stromal cells also express osteoprotegerin OPG, which competes with RANK for binding to RANKL, blocks the induction of osteoclasts and inhibits bone resorption. NFATc1 is a downstream factor of RANK signaling, which promotes the expression of osteoclast-specific genes against tartrate acid phosphatase TRAP and calcitonin receptor, leading to terminal differentiation of osteoclasts. Isorhamnetin promotes osteoprotegerin OPG expression in bone tissue, inhibits RANK/RANKL signaling pathway activation, thereby reducing the expression of NFATc1 resulting in a decrease in TRAP and osteocalcin (OC) expression. This regulates the function of osteoblasts and osteoclasts, inhibits bone destruction and improves bone microstructural damage in ovariectomized rats. Thus isorhamnetin can be used in the prevention and treatment of osteoporosis. Li et al. [73] found that isorhamnetin could inhibit the differentiation of RAW264.7 cells into osteoclasts and the mechanism was related to inhibition of the classical NF-κB pathway. The NF-κB pathway may be the main one by which isorhamnetin inhibits bone destruction and displays anti-osteoporosis properties. The anti-osteoporosis effect and mechanism of isorhamnetin are shown in Table 4.

Table 3
Anti-inflammatory effect of isorhamnetin and the related mechanism.

Therapeutic effect	Experimental subject	Observation	Action	Mechanism of action	References
Anti-acute lung injury	Intratracheal infusion of LPS (3 mg / kg) in male BALB/c mice	In vivo 60 mg/kg	Anti-inflammation, antioxidation	Inhibition of COX-2 expression	[60]
Anti-acute lung injury	LPS-stimulated RAW264.7 mouse macrophages and male BALB/ c mice	In vitro 0 ~ 20 μg/mL In vivo 30, 60 mg/kg	Anti-inflammation	Inhibition of MAPK and NF- kappa B pathways and preparation of phosphorylation of ERK,JNK, I κ Ba and NF- κ B (p65) activated by LPS in vivo	[59]
Anti-acute lung injury	LPS-stimulated RAW264.7 mouse macrophages and male BALB/ c mice	In vitro 0–2 nM In vivo 6, 12, 24 nM	Anti-inflammation	Blocking the activation of NF- kappa B signal and down-regulating the secretion of pro-inflammatory cytokines (TNF-1,IL-1,IL-6)	[61]
Anti-periodontitis	Human gum fibroblasts stimulated by LPS	In vitro 10,20,40μM	Anti-inflammation	Activation of Nrf2 signaling pathway, up-regulation of the expression of Nrf2 and HO-1, and inhibition of the activation of NF-κB	[65]
Anti-inflammation bowel disease	Human colon cancer cell line HT-29 and LS174 T and mouse macrophage strain RAW264.7	In vitro 1, 10, 25 μM In vivo 20 mg/kg	Anti-inflammation	Activation of PXR, promotion of the up-regulation of PXR-mediated metabolism of probiotics and the down-regulation of NF- kappa B signal transduction	[66]
Anti-bv2 microglial inflammation	LPS-stimulated BV2 mouse microglia	In vitro 50, 100, 200 μM	Anti-inflammation	Inhibition of NF- kappa B signaling pathway, antagonism of TLR4 and elimination of ROS accumulation	[67]
Anti-osteoarthritis	Human cartilage cells stimulated by IL-1 β	In vitro 10, 50, 100 μg/mL	Anti-inflammation, cartilage protection	Inhibition of the expression of NF-κB and transcription factor p65, and reduction of the degradation of NF-κB inhibitor α in cartilage cells induced by IL-1 β	[68]
Anti-pulmonary tuberculosis	Human lung fibroblasts (MRC-5 cells)stimulated by IFN- γ	In vitro	Anti-inflammation	Decrease of mRNA expression of TNF α, IL-1 β, IL-6,IL-12 and matrix metalloprotease-1	[62]
Anti-acute kidney injury	Ischemia-reperfusion injury model in male SD rats	In vivo 50 mg/(kg·d), 150 mg/(kg·d)	Anti-inflammation, antioxidation	Inhibition of excessive activation of NF- kappa B signaling pathway, reduction of inflammatory reaction and oxidative stress	[63]
Kidney protection	Type 2 diabetic rats induced by high fat diet and intraabdominal injection of streptozotocin	In vivo 50,150 mg/kg/day	Anti-inflammation, antioxidation	Inhibition of NF- kappa B signal transduction activity, reduction of inflammatory mediators and decrease of oxidative stress	[64]

8. Anti-oxidation

Isorhamnetin can scavenge DPPH radical and ABTS radical and inhibit lipid peroxide of liver mitochondria *in vitro*, showing an antioxidant activity [74]. Isorhamnetin has a potential protective effect against oxidative stress in human RPE cells, and the mechanism of action is related to the activation of PI3K/Akt signal transduction pathway. Thus, it may be considered as a potential antioxidant for the prevention of age related macular degeneration [75]. Isorhamnetin can affect mitochondrial apoptosis pathway and activate Nrf2/ ARE signaling pathway to realize antioxidant and anti-apoptotic functions, protecting rat cardiomyocytes (H9C2) from hydrogen peroxide damage [8]. Isorhamnetin can strengthen the cellular antioxidant defense capacity by activation of the Nrf2/ HO-1 and ERK pathways, thus preventing C2C12 cells from H_2O_2-induced cytotoxicity [76]. Seo et al. [77] found that isorhamnetin inhibited the migration and invasion of cancer cells *in vitro* through inhibition of HIF-1α. Isorhamnetin also has an antioxidant effect on linoleic acid peroxide induced by Gu^{2+} and H_2O_2 [78]. These suggest that the anti-cancer effect of isorhamnetin is relevant to anti-oxidation.

Kong et al. [79] evaluated the inhibitory effect of isorhamnetin 3-O-β-D-glucopyranoside on oxidative stress in free cells and cell systems. It not only exhibited dose-dependent scavenging activities on the generation of DPPH, hydroxyl and carbon-centered radicals, but also decreased the intracellular ROS level. It also increased the expression of glutathione (GSH) and antioxidant enzymes, and suppressed the oxidative damage of purified genomic DNA and the activity of MPO in human bone marrow cells induced by TNF-α. Isorhamnetin is possibly a candidate worthy of being developed as a natural antioxidant. In addition, some studies have shown that isorhamnetin can inhibit the oxidative modification of HDL [80,81], LDL [82] and VLDL [83] induced by Cu^{2+}.

In a word, isorhamnetin displays antioxidant properties by activating PI3K/Akt signal transduction pathway and Nrf2/ ARE signal transduction pathway to inhibit apoptosis (as shown in Table 4).

9. Effect on hepatocytes

AMPK, an AMP-dependent protein kinase, plays an important role in the regulation of cellular energy homeostasis, which can be activated by CAMKK2. Isorhamnetin inhibits ROS generation, mitochondrial dysfunction and GSH decrease induced by arachidonic acid (AA) + iron [84], suggesting that isorhamnetin is probably a potential candidate drug for the prevention of liver disease (Table 4). Isorhamnetin represses HSC activation through activating Nrf2-ARE signaling, inhibiting TGF-β-mediated ROS production, and subsequently suppressing classical TGF-β/Smad signaling pathway. This leads to the inhibition of fibrosis gene expression including α-SMA and PAI-1, thereby exerting an inhibitory effect on HSC activation, and consequently liver fibrosis is prevented [85].

P38 is a downstream factor of MAPKs signaling pathway, which can inhibit the activation of toll-like receptors to regulate the mRNA levels of PPARs, and PPAR-α is an isoform of PPARs, which is closely related to cell apoptosis and autophagy [86]. Isorhamnetin can inhibit ConA-induced acute fulminant hepatitis (AFH) in mice through inhibiting apoptosis and autophagy via the mouse P38/PPAR-α pathway [87]. It can decrease the content of MDA, increase the content of GSH, ehance the activity of SOD and GSH-Px activity and lower the release of ALT and AST, protecting the damage of human normal hepatocytes (LO2) induced by paracetamol (APAP) [88]. However, the specific mechanism of action is unclear and needs further research. Fig. 6 shows the protective mechanism of isorhamnetin on liver.

10. Anti-hypoxia

Jiang et al. [89] studied the effects of isorhamnetin on the survival

Table 4
Other pharmacological action and mechanism of isorhamnetin.

Therapeutic effect	Experimental subject	Observation	Action	Mechanism of action	References
Anti-pulmonary fibrosis	C57 mouse lung fibrosis model (intraperitoneal injection of bleomycin 3.5 U/ kg) and human A549 cells and human bronchial epithelial cells stimulated by TGF-1	In vivo 10, 30 mg/kg In vitro 25, 50, 100 μM	Inhibition of endoplasmic reticulum stress and collagen deposition, and reduction of expression of type I collagen and alphasma	Inhibition of Perk signal activation	[7]
Resistance to endoplasmic reticulum stress injury	N2a cell line	In vitro 10, 20, 40μM	Inhibition of Ca^{2+} overload, reduction of ROS production and decrease of apoptosis	Promotion of the phosphorylation of pkc- ε and activation of the pkc- ε pathway	[70]
Anti-osteoporosis	Rat femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues	In vitro 10^{-7}-10^{-5}M	Inhibition of osteoclast formation and bone resorption	Undefined	[71]
Anti-osteoporosis	Ovariectomy SD rats	In vivo 30 mg/(kg·d)	Regulation of the function of osteoblasts and osteoclasts and improvement of bone microstructure damage	Regulation of the RNAKL/ RNAK/ OPG signaling pathway	[72]
Anti-osteoporosis	RAW264.7 cells	In vitro 1～10μM	Inhibition of differentiation of RAW264.7 cells into osteoclasts	Inhibition of classical NF-κB pathway and decrease of mRNA expression of TRAP, Ctsk, MMP-9, c-Fos, NFATc1 and NF-κB p65	[73]
Antioxidation	Rat liver mitochondria	In vitro 32, 14. 54, 6.67 μmol /L	Inhibition of liver Mitochondrial Lipid Peroxidation	Undefined	[74]
Prevention of age-related macular degeneration	Human retinal pigment epithelial cell line Arpe-19	In vitro 25, 50, 100 μM	Anti-oxidative stress of human retinal pigment epithelial cells	Activation of PI3K / Akt signaling pathway	[75]
Antioxidant stress	C2C12 myoblasts stimulated by H_2O_2	In vitro 20, 30 μM	Enhancement of the antioxidant defense ability of cells	Activation of NRF2/HO-1 pathway (including activation of ERK pathway)	[76]
Prevention of hepatotoxicity of AA plus iron	HepG2 (human), AML12 (mouse), and H4IIE (rat) hepatocyte derived cell lines	In vitro 3, 10, 30, 100μM	Antioxidation	Activation of AMPK-CAMKK2 pathway to inhibit reactive oxygen species production, mitochondrial dysfunction and decrease of glutathion levels	[84]
Anti-hepatic fibrosis	LX-2 cells stimulated by TGF- β1 and Male ICR mice stimulated by CCl4	In vitro 25–100μM In vivo 10, 30 mg/ kg	Antioxidation	Inhibition of TGF- β / Smad signaling pathway and activation of hepatic stellate cells	[85]
Anti-acute fulminant hepatitis	Intraabdominal injection of Male Balb/C mice with concanavalin A (25 mg / kg)	In vivo 10, 30, 90 mg/kg	Inhibition of apoptosis and autophagy	Inhibition of P38 / PPAR- α pathway, inhibition of p38 phosphorylation and promotion of PPAR- α expression	[87]
Anti - human normal hepatocyte (L02) injury	L02 cells stimulated by paracetamol	In vitro 5, 10, 20 μmol /L	Enhancement of the survival rate of injured cells, antioxidation	Decrease of the content of MDA, increase of the content of GSH, the activity of SOD,GSH-Px, and reduction of the release of ALT and AST	[88]
Anti-hypoxia	Normobaric hypoxia mice, specific myocardial anoxic mice, sodium nitroso poisoning mice	In vivo 1, 2, 5 g/kg	Improvement of hypoxia tolerance, reduction of oxygen consumption and increase of survival time	Undefined	[89]
Anti-hyperuricemia	AML12 cells, purine induced hyperuricemia in mice	In vitro 10, 20, 30μM In vivo 100, 300 mg/kg	Reduction of plasma and liver UA levels	Inhibition of XO activity and UA production in liver	[6]
Regulating immunity	bone marrow-derived dendritic cell	In vitro 10, 10, 500 μM	Inhibition of DC activation and transport	Down-regulation of TNF-α, IL-6, IL-1 β and IL-12p70, inhibition of CD40, CD80, CD86 and chemokine receptor 7	[91]
Anti-influenza virus	Female C57BL/6 mice, MadinDarby dog kidney (MDCK) cells	In vivo 1 mg/kg/day In vitro 10, 50, 100 μM	Inhibition of virus - induced autophagy, ROS production, and ERK phosphorylation.	Inhibition of HA and NA gene expression	[92]
Anti-Staphylococcus aureus	S. aureus strains ATCC 29212, ATCC 10832, USA 300, 8325-4, DU 1090, and human alveolar epithelial cell line A549	In vitro 2, 4, 8, 16 μg/mL.	Anti-α–hemolysin	Downregulation of RNA III expression and inhibition of alpha-hemolysin HLA transcription	[93]
Anti-bacterial	Escherichia coli, Klebsiella pneumoniae, Proteus, Staphylococcus aureus and Bacillus	In vitro	Anti-bacterial	Undefined	[95]

(*continued on next page*)

Table 4 (*continued*)

Therapeutic effect	Experimental subject	Observation	Action	Mechanism of action	References
Anti-vitiligo	B16F10 melanoma cell line	In vitro 8, 16, 32 μM	Promotion of melanin production	The targeting of MC1R-MITF signaling pathway, MAPK signal pathway, PPAR signaling pathway (PPARA, PPARD, PPARG), arachidonic acid metabolic pathway (ALOX12, ALOX15, CBR1) and 5-hydroxytryptamine synapse (ALOX12, ALOX15) effectively increased melanogenesis	[97]
Prevention of obesity	Dietary obese mouse model and isolated islets	In vitro In vivo	Increase of insulin secretion and energy consumption in mice fed with a high-fat diet	Promotion of insulin secretion and up-regulation of CPT-1 to increase energy consumption, reduce adipocytes size and body weight, and inhibit oxidative stress and hepatic steatosis	[98]
Prevention of obesity	3T3-L1 preadipocytes and female C57/BL6 mice	In vitro 10, 25, 50μM In vivo 100 mg/(kg·d)	Reduction of body weight, improvement of insulin resistance, and liver fat degeneration	Antagonizing PPAR γ and inhibiting the activity of PPAR γ	[99]
Prevention of obesity	3T3L1 cells	In vitro 0.1, 0.5, 1, 10, 20, 50 μM	Inhibition of fat formation and the promotion of mitochondrial biogenesis	Promotion of AMPK phosphorylation, inhibition of GPDH, PPAR γ, aP2, reduction of fat production, up-regulation of PGC-1 α, NRF1, Tfam, promotion of mitochondrial biogenesis	[100]
Anti-UV damage	Human HaCaT keratinocytes	In vitro 2.5, 5, 10, 20 μM	Inhibition of apoptosis and mitochondrial dysfunction promoted by UVB	Undefined	[101]

time of mice under different hypoxia conditions (as shown in Table 4). Experimental animal models of atmospheric hypoxia, specific myocardial hypoxia and sodium nitroso toxic hypoxia were established by different methods. The results showed that isorhamnetin could significantly prolong the survival time of normobaric hypoxia, specific myocardial hypoxia and sodium nitroso toxic hypoxia in mice, indicating that isorhamnetin can enhance the hypoxia tolerance activity in animals. However, the mechanism of action needs verification further.

11. Anti-hyperuricemia

Hyperuricemia is an important risk factor for gout, which not only causes damage to renal function, but also induces cardiovascular and cerebrovascular diseases, and is accompanied by serious complications. Isorhamnetin can decrease the activity of xanthine oxidase (XO) and inhibit the production of uric acid (UA) in a dose-dependent manner without changing the expression of XO protein in the liver. These findings demonstrate that isorhamnetin has a potent anti-hyperuricemic effect and may be a potential candidate for prevention and remediation of hyperuricemia [6]. The anti-hyperuricemia effect of isorhamnetin and involved mechanism are shown in Table 4.

12. Regulating immunity

Wang et al. [90] found that isorhamnetin, the main component of Miaoyao Fanggan sachet (MFS), could enhance innate immunity when they studied the effect of MFS on the response of the innate immune system. Dendritic cells (DCs) are regarded as important targets for immunosuppressive therapy because of their important role in linking natural and adaptive immunity. Shi et al. [91] investigated the function and mechanism of isorhamnetin on BMDCs including maturation, phagocytosis, and trafficking. It was found that isorhamnetin effectively inhibited the maturation of LPS-treated BMDCs by down regulation of TNF-α, IL-6, IL-1beta and IL-12p70, up regulation of IL-10, and depression of CD40, CD80, and CD86, but no effects on phagocytosis. In addition, chemokines are a class of small molecule basic proteins whose main function is the directional movement of chemotactic cells. Isorhamnetin depressed the migration of LPS-treated BMDCs possibly through inhibition of CCR7 expression. Isorhamnetin might be also a potent immunosuppressive agent, which inhibited the activation and transport of dendritic cells to reduce inflammatory responses. The effect of isorhamnetin on immune regulation and involved mechanism are shown in Table 4.

13. Anti-bacterial and anti-viral effects

Ahmed et al. [92] found isorhamnetin to be an effective antiviral agent. In mice infected with the influenza A virus, isorhamnetin significantly reduced lung virus titer by 2 folds, raised the survival rate ranging from 70 to 80%, and decreased body weight loss by 25 %. The methyl group located on the B ring of isorhamnetin may contribute to its strong antiviral potency against influenza virus in comparison with other flavonoids. This anti-influenza effect is related to the inhibition of HA and NA gene expression, virus-induced autophagy, ROS production and ERK phosphorylation. Jiang et al. [93] found that isorhamnetin was able to downregulate the gene RNAIII encoding alpha-hemolysin (Hlα) and inhibiting Hlα transcription. In a co-culture system for S. aureus and lung cells, topical isorhamnetin treatment protected against cell injury elicited by S. aureus. Isorhamnetin may be a leading compound for the development of anti-virulence drugs against S. aureus infections [93]. When investigating antibacterial activity mechanism of polyphenols, Bhattacharya et al. [94] found that isorhamnetin had the ability to permeate bacterial cell membrane through oxidative stress, indicating its potential antibacterial activity which was further confirmed by Habtamu and Melaku [95]. Although the mechanism of

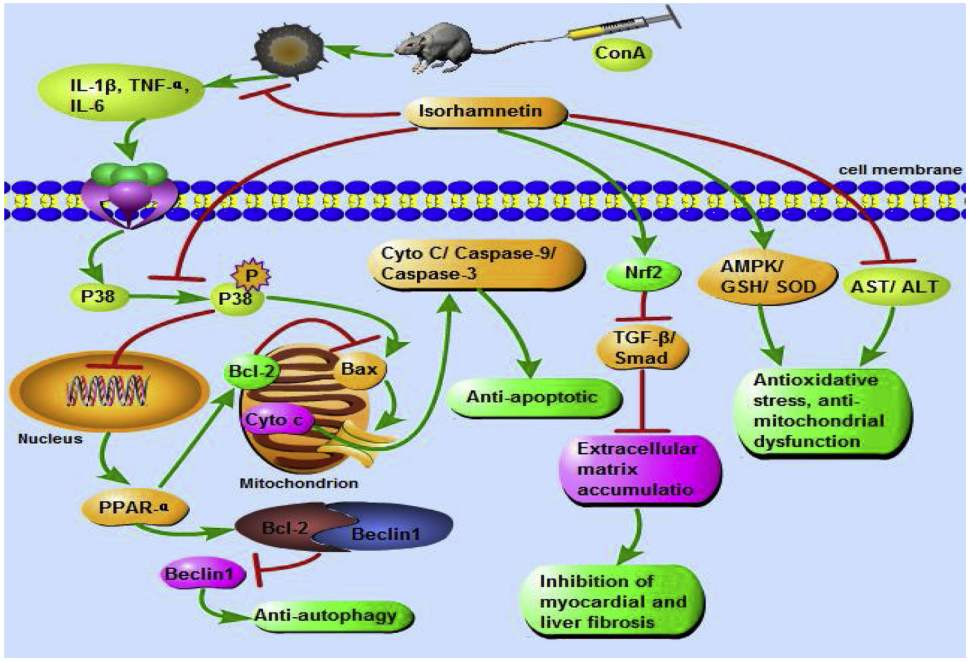

Fig. 6. Protective mechanism of isorhamnetin on liver. Isorhamnetin can protect the liver from many injuries such as autophagy and apoptosis by regulating a variety of signal pathways.

action needs to be determined further. The anti-bacterial and anti-viral effects and mechanism of isorhamnetin are shown in Table 4.

14. Treatment of vitiligo

Vitiligo is a common acquired localized or generalized skin pigmentation disease and the current treatment is limited. Studies have shown that all three PPAR subtypes are expressed in melanocytes, which can promote melanin production [96]. The arachidonic acid metabolic pathway and serotonin synapses are present in keratinocytes and pigment cells, which promote the secretion of regulatory factors such as PGE2, HETE and 5-HT. These regulators stimulate melanocyte proliferation to produce melanin. Wang et al. [97] successfully predicted the melanogenic activity of isorhamnetin from *Vernonia anthelmintica* (L.) through admetSAR and SDTNBI methods of network pharmacological analysis. It significantly increased the tyrosinase activity, MITF protein expression and melanin-biosynthetic genes (MC1R, MITF, TYR, TYRP1 and DCT) mRNA-expression. Based on the SDTNBI method and experimental verification, isorhamnetin effectively increased melanogenesis by targeting serotonergic synapses (ALOX12, ALOX15) and pathways of MC1R-MITF, MAPK, PPAR (PPARA, PPARD, PPARG), and arachidonic acid metabolism (ALOX12, ALOX15, CBR1) (Table 4).

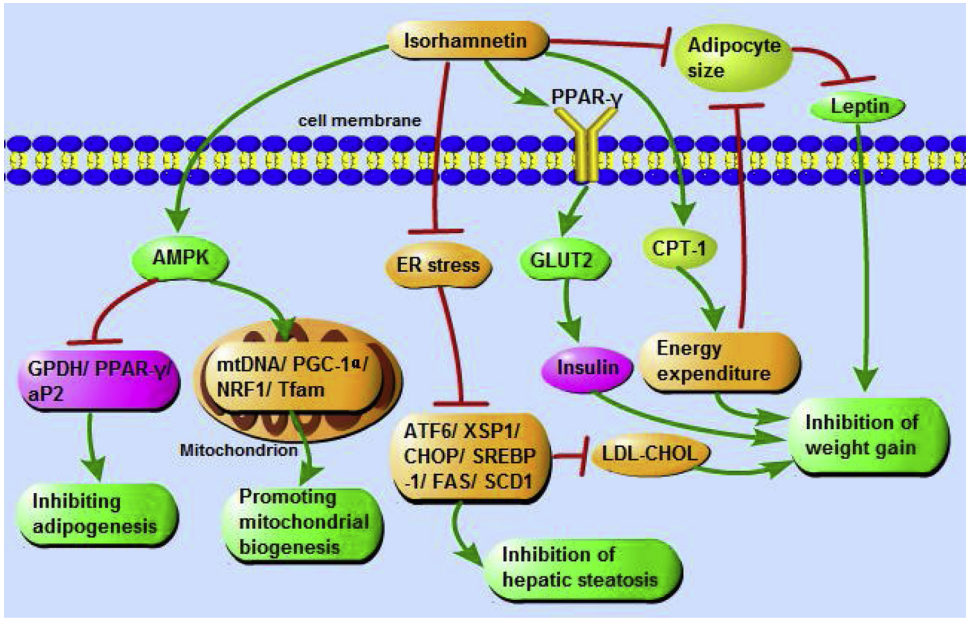

Fig. 7. Obesity-preventing mechanism of isorhamnetin. Isorhamnetin can prevent obesity by inhibiting adipogenesis and promoting mitochondrial biogenesis.

15. Prevention of obesity

César et al. [98] found that isorhamnetin extract from opuntia ficus-indica (OFI) could reduce weight gain, increase insulin secretion and energy consumption in mice fed with high-fat diet. This was eventually responsible for the decrease of fat accumulation in liver and adipose tissue, thus preventing hepatic steatosis and lipocyte hypertrophy. Therefore, obesity may be prevented by ingestion of OFI containing isorhamnetin (as shown in Table 4).

PPAR-γ activation may induce obesity and NAFLD. Isorhamnetin is a novel antagonist of PPAR-γ, which suppresses the adipocyte differentiation elicited by the PPAR-γ agonist rosiglitazone, obstructs obesity development and alleviates hepatic steatosis caused by both high-fat diet and leptin deficiency [99]. The molecular mechanism of action involves isorhamnetin-mediated mitochondrial biogenesis and AMPK activation in 3T3-L1 cells [100]. The mitochondrial biogenic effect of isorhamnetin in adipocytes might be related to stimulation of mitochondrial gene expression, mtDNA replication, and AMPK activation. Fig. 7 shows the obesity-preventing mechanism of isorhamnetin.

16. Anti-ultraviolet injury

Han et al. [101] found that isorhamnetin eliminated ultraviolet (UV) B-induced intracellular ROS and attenuated the oxidative modification of DNA, lipids and proteins in response to UVB radiation (as shown in Table 4). Furthermore, it inhibited the programmed cell death of keratinocytes promoted by UVB. Additionally, isorhamnetin inhibited mitochondrial dysfunction induced by UVB light, protecting human keratinocytes from UVB-induced cell injury and death. Although the effect is related to the antioxidant property of isorhamnetin, the mechanism of action remains to be determined.

17. Conclusions and perspectives

Isorhamnetin has extensive pharmacological effects. Anti-osteoporosis, immune regulation and other pharmacological effects and the involved mechanisms are shown in Table 4. The mechanisms of action mainly involve anti-inflammation, antioxidation and regulation of apoptosis. These pharmacological activities often play an important role in the treatment of different diseases, such as the anti-oxidative and anti-inflammatory effects of isorhamnetin on acute kidney injury and acute fulminant hepatitis in mice. At the same time, isorhamnetin can inhibit HIF-1α due to its antioxidant potential, thus inhibiting the migration and invasion of cancer cells *in vitro*. The multiple effects of isorhamnetin play an important role in the treatment of diseases by PI3K/AKT, NF-κB and other signaling pathways and cytokines, displaying a high medicinal value.

However, this review points out some limitations of the pharmacological research on isorhamnetin. i) At present, there are many *in vitro* investigations on isorhamnetin, but relatively few *in vivo* studies; ii) The toxicological study is insufficient; iii) The mechanisms of anti-hypertensive, antithrombotic, anti-hypoxia and anti-ultraviolet damage have not been elucidated; iiii) Few therapeutical targets are investigated for isorhamnetin, which of is great significance to clarify the exact mechanism of action of isorhamnetin.

In future, it is necessary to focus on the following aspects: i) strengthening *in vivo* research, especially the effects on PI3K/ AKT, NF-κB signaling pathways and cytokines, and further exploring the pharmacological action and related molecular mechanism; ii) Strengthening the study of toxicology and drug interactions of isorhamnetin to establish the safety characteristics of humans and promote the development of medicinal value of isorhamnetin; iii) Strengthening the study on pharmacological effects and structure-activity relationship of isorhamnetin and its derivatives; iiii) Investigating the binding target for pharmacological effects of isorhamnetin. Finally, it is hoped that further research will address the concerns raised above to provide further data so that isorhamnetin can be clinically used for the treatment of diseases.

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Declaration of Competing Interest

The authors declare that there are no competing interests associated with the manuscript.

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