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REVIEW

# Development of Chinese herbal medicine for sensorineural hearing loss



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Received 22 May 2023; received in revised form 16 September 2023; accepted 24 October 2023

## KEY WORDS

Sensorineural hearing loss;  
Chinese herbal medicine;  
Molecular mechanism;  
Active ingredients;  
Prescriptions;  
Ototoxic drugs;  
Noise;  
Hair cells

**Abstract** According to the World Health Organization's world report on hearing, nearly 2.5 billion people worldwide will suffer from hearing loss by 2050, which may contribute to a severe impact on individual life quality and national economies. Sensorineural hearing loss (SNHL) occurs commonly as a result of noise exposure, aging, and ototoxic drugs, and is pathologically characterized by the impairment of mechanosensory hair cells of the inner ear, which is mainly triggered by reactive oxygen species accumulation, inflammation, and mitochondrial dysfunction. Though recent advances have been made in understanding the ability of cochlear repair and regeneration, there are still no effective therapeutic drugs for SNHL. Chinese herbal medicine which is widely distributed and easily accessible in China has demonstrated a unique curative effect against SNHL with higher safety and lower cost compared with Western medicine. Herein we present trends in research for Chinese herbal medicine for the treatment of SNHL.

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Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2023.11.001>

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and elucidate their molecular mechanisms of action, to pave the way for further research and development of novel effective drugs in this field.

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## 1. Introduction

Hearing loss is one of the most frequent chronic diseases, over 466 million people worldwide (5.5% of the population) have disabling hearing loss. By 2050, it is estimated that one in four people will have hearing problems, and about 2.5 billion people will have varying degrees of hearing loss, of which at least 700 million people will need rehabilitation services (World Health Organization, 2021). Hearing loss not only affects the physical and mental health of individuals, but also wreaks havoc on the national economies. Unemployment rates of people with hearing loss are higher than those with normal hearing in both developed and developing countries. Nowadays there are still no approved effective agents available as yet except devices such as cochlear implants and hearing aids. Therefore, drug therapies for the prevention and treatment of hearing loss must be developed for human use.

Sensorineural hearing loss (SNHL) is the most common sensory deficit and typically occurs due to damage to the cochlear sensory hair cells, stria vascularis, or the synapse with primary auditory neurons in inner ears<sup>1–3</sup>. The etiology of SNHL is multifactorial and complicated which may be attributed to ototoxic drugs such as aminoglycoside antibiotics and platinum drugs, exposure to loud noise with prolonged periods, the aging process, or infection<sup>4–7</sup>. The underlying mechanisms of SNHL are well investigated in recent years in order to seek novel therapeutic strategies. Currently, steroid therapy has been considered the most widely used treatment for SNHL clinically by immunosuppression and anti-inflammatory action<sup>8,9</sup>. However the therapeutic effect of steroid therapy remains debatable and there are also lots of side effects such as gastrorrhagia, hypertension, infection, and hepatic dysfunction.

As a treasure of the Chinese nation, Chinese herbal medicine (CHM) has developed for thousands of years. CHM refers to a natural source that has not been processed or is only simply processed and used for disease prevention and medical care under the guidance of traditional Chinese medicine theory. Recently, with the acceleration of the modernization of CHM, the advantages of CHM and its active components in the prevention and treatment of multiple diseases become more obvious. Substantially different from other single small molecule drugs, CHM including formula prescriptions and natural products works by symptomatic treatment through various ways and multiple targets, emphasizing the significance of enhancing the individual's endogenous healing ability from a holistic and natural view<sup>10</sup>. In addition to being able to treat diseases, CHM also plays a pivotal role in health care and preservation<sup>11</sup>. Although CHM is confronted with problems such as unclear therapeutic substance basis and mechanism, difficult quality control, registration/policy barriers, and lack of intellectual property rights, its curative effect is remarkable with fewer side effects and lower cost. This review

will provide an overview of the prophylaxis and treatment of SNHL with CHM and address new drugs that have potential therapeutic effects.

## 2. Cause and pathophysiological mechanism of SNHL

### 2.1. Ototoxic drugs-induced hearing loss

Several drugs can cause cochlear hair cell damage and lead to hearing loss, the most commonly encountered ototoxic drugs are the aminoglycosides and the antineoplastic agent cisplatin. Aminoglycoside antibiotics, including streptomycin, neomycin, gentamicin, kanamycin, and so on, are a kind of glycoside antibiotics formed by connecting amino sugar and amino cyclic alcohol through an oxygen bridge<sup>12</sup>. They are mainly employed to prevent and treat various systemic infectious diseases in the clinic and can infer acute kidney injury and irreversible hearing loss<sup>13</sup>. It has been reported that the main mechanism of ototoxicity caused by aminoglycosides is that aminoglycosides enter the mitochondria of vestibular and cochlear hair cells through ion channels or transporters and accumulate, further inhibiting the synthesis of mitochondrial proteins and oxidative phosphorylation of vestibular and cochlear hair cells, resulting in mitochondrial dysfunction, and finally leading to hair cell apoptosis and hearing loss<sup>14–16</sup>.

Platinum drugs such as cisplatin, is a chemotherapeutic agent currently prescribed for the treatment of several forms of human cancer. Cisplatin can cause damage to cochlear hair cells and thus lead to hearing loss. Studies have shown that the ototoxicity of cisplatin may be related to the increased reactive oxygen species (ROS) formation and oxidative decompensation in mitochondria<sup>17–19</sup>. Permanent ototoxicity induced by cisplatin requires cisplatin to enter cochlear hair cells. Multiple evidence suggests that cisplatin preferentially enters the endolymph in the inner ear through the stria vascularis by copper-like transport-1 and organic cation transporter-2<sup>20–23</sup>. Then it enters the hair cell cytoplasm and leads to the depletion of glutathione and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase), resulting in the increase of oxidative stress and promoting apoptotic and necrotic cell death<sup>17,24</sup>.

### 2.2. Noise-induced hearing loss

Frequent exposure to excessive recreational, social, and residential noise may affect auditory function. As is reported that acoustic overexposure may increase auditory brainstem response (ABR) threshold shifts, accompanied by loss of cochlear hair cells and synaptic ribbons<sup>25–27</sup>. It is currently believed that excessive noise stimulation can lead to the formation of free radicals, ROS, or

excitatory toxicity of glutamate, which in turn activates intracellular signaling pathways leading to cell death. ROS generated by noise exposure can also activate nuclear factor-kappa B (NF- $\kappa$ B), cause the production of proinflammatory cytokines, and lead to hearing impairment<sup>28</sup>. The accumulation of ROS in cells will destroy the cell structure, form damaged organelles and misfolded proteins, activate the occurrence of autophagy, and cause cell death when cells are severely damaged, thus leading to hearing loss<sup>29,30</sup>. Furthermore, noise-induced Ca<sup>2+</sup> overload can also trigger apoptosis and necrotic cell death pathways independently of ROS formation<sup>31</sup>. Noise exposure activates AMPK through LKB1-mediated pathways to induce hair cell death and synaptic loss<sup>32</sup>. In addition, extracellular ATP-activated ion channels which are non-selective cation channels expressed in hair cells and squamous epithelial cells of the cochlea can also mediate noise-induced hearing loss<sup>33</sup>.

### 2.3. Presbycusis

Age-related hearing loss, termed presbycusis, is a complex degenerative disease. With the increase of age, hearing decreases symmetrically and progressively, which is characterized by the decline of hearing function, including the increase of hearing threshold and the decrease of frequency<sup>34</sup>. Presbycusis is the third most common health problem among the elderly, second only to heart disease and arthritis. About two-thirds of people aged 70 or over have hearing impairment<sup>35,36</sup>. Loss of cochlear hair cells and degeneration of spiral ganglion are the main pathological features of presbycusis<sup>37</sup>. Compared with the young cochleae, the aging cochleae exhibit severe synapse and nerve fiber loss, significant pro-inflammatory and necrotic reactions, and decreased mitochondria level<sup>38</sup>. It also has been reported that when presbycusis occurs, the mitochondrial oxidative damage in marginal cells of stria vascularis reduces the generation of adenosine triphosphate, which in turn reduces sodium ions, potassium ions, and the activities of adenosine triphosphate enzymes, resulting in a decrease in endocochlear potential and an increase in hearing threshold<sup>39</sup>.

### 2.4. Others

Hereditary hearing loss which results from genetic factors is also devastating. More than 700 thousand babies are born with permanent hearing loss annually in low- and middle-income countries. Hereditary hearing loss can be divided into two types: nonsyndromic hearing loss and syndromic hearing loss. Among these 70% is nonsyndromic hearing loss, and the clinical manifestations are mainly bilateral symmetrical hearing loss without other symptoms; Syndromic hearing loss accounts for about 30% of congenital hearing loss with other congenital malformations<sup>40</sup>. The majority of hereditary hearing loss is related to a few gene mutations, such as *GJB2*, *SLC26A4*, *OTOF*, *MYO15A*, and *TMC1*<sup>41,42</sup>. Hearing loss can also result from injuries and infections. Traumatic brain injury which results from a bump, jolt, or penetrating injury to the temporal bone may disrupt the anatomic structures of the inner ear thus leading to hearing loss<sup>43</sup>. Cytomegalovirus is the most common pathogen that causes congenital infection in neonates. Amounts of studies show that cytomegalovirus is the main cause of hearing loss and nervous system diseases in children<sup>44–46</sup>. Mumps is an acute infectious disease caused by the mumps virus, which can cause hearing loss by affecting the cochlea and auditory nerve<sup>47</sup>. Other viral

infections including rubella, measles, and human immunodeficiency virus can also be the cause of congenital or acquired hearing loss.

## 3. CHM treatment for SNHL

The etiology and pathogenesis of SNHL are complicated, which may be a manifestation of either a single symptom or multiple disease syndromes. In traditional Chinese medicine, hearing loss is closely associated with the dysfunction of other organs such as the kidney, and many herbal species have been used for the nourishment of the kidney and other organs to treat deficiencies in hearing. CHM treatment is a conventional therapy for SNHL and can produce unexpected and unique curative effects.

A great deal of studies have been carried out to find anti-SNHL CHM in the past few decades. Here we overviewed the CHM including active ingredients, single CHM extracts, and CHM prescriptions, that exhibited significant protective effects against hearing loss in preclinical (Table 1) and clinical (Table 2) trials.

### 3.1. Active ingredients of CHM

The major active components are the pharmacodynamic material basis of CHM. The identified anti-SNHL active ingredients which showed anti-inflammatory, anti-oxidative, anti-apoptotic, and neuroprotective effects could be generally divided into triterpenoids, polyphenols, flavonoids, terpenoids, and saccharides (Fig. 1).

#### 3.1.1. Triterpenoids

Triterpenoids are extensively distributed in nature, some of them exist in plants in free form, called triterpenoid sapogenins. Some exist in the form of glycosides by combining with sugars, called triterpenoid saponins. Triterpenoids have a wide range of physiological properties, such as hypoglycaemic<sup>48</sup>, immunoregulatory<sup>49</sup>, anti-cancer<sup>50</sup>, and anti-inflammatory<sup>51</sup> activities. Triterpenoid saponins have been highlighted for their neuroprotective effects<sup>52</sup>. Astragaloside IV, which is the major active component of the Chinese herb *A. membranaceus*, shows a significant protective effect against noise-induced cochlear damage. By oral administration of astragaloside IV, increased levels of iNOS and nitrotyrosine and apoptotic marker active caspase 3 induced by impulse noise were effectively reduced<sup>53,54</sup>. Ursolic acid is a triterpene compound derived from *C. officinalis* with significant antioxidant effects<sup>55</sup>. It has been reported that ursolic acid treatment attenuates cisplatin-induced ototoxicity through inhibiting the activation of the TRPV1-oxidative stress pathway<sup>56</sup>. And pre-treatment with ursolic acid (0.05–2  $\mu$ g/mL) could effectively protect against hydrogen peroxide-induced auditory House Ear Institute-Organ of Corti 1 (HEI-OC1) cell damage and reverse the decrease of catalase and glutathione peroxidase activities<sup>57</sup>. Ginsenoside is the most important active ingredient in *P. ginseng* and exerts strong antioxidant, anti-tumor, anti-aging, and neuroprotective effects<sup>58</sup>. Studies showed that ginsenoside Rb1 could protect against gentamicin-induced vestibular/hearing dysfunction by reducing reactive oxygen species production and inhibiting apoptotic pathways<sup>59</sup>, and attenuated cochlear spiral ganglion cell injury caused by ischemic insult<sup>60</sup>. Ginsenoside Rd, another ginsenoside compound, also exhibits otoprotective effects against noise-induced hearing loss mainly by activating the SIRT1/PGC-1 $\alpha$  signaling pathway<sup>61</sup>.

**Table 1** Effects of CHM in animal models of SNHL.

CHM			SNHL Model	Effects	Ref.
Active ingredients of CHM	Triterpenoids	Astragaloside IV	Noise-induced hearing loss in guinea pigs	Reducing iNOS, nitrotyrosine, and active caspase 3	48–54
		Ursolic acid	Cisplatin-induced ototoxicity in BALB/c mice	Inhibiting TRPV1-oxidative stress pathway	55–57
		Ginsenoside Rb1	Gentamicin-induced hearing loss in SD rats; cochlear ischemia-induced hearing loss in Mongolian gerbils	Reducing ROS and inhibiting apoptosis	58–60
		Ginsenoside Rd	Noise-induced hearing loss in guinea pigs	Alleviating apoptosis, oxidative stress, and activating SIRT1/PGC-1 $\alpha$ signaling pathway	61
	Polyphenols	Rosmarinic acid	Cisplatin-induced ototoxicity in rat explants; noise-induced hearing loss in Wistar rats	Inhibiting apoptosis and reducing ROS; potentiating Nrf2–ARE signaling pathway	62–64
		Curcumin	Gentamicin and sodium salicylates-induced ototoxicity in SD rats	Inhibiting oxidative stress, apoptosis, and inflammation	65–70
		Resveratrol	Cisplatin-induced ototoxicity in mice; kanamycin and furosemide-induced ototoxicity in Wistar rats; noise-induced hearing loss in C57BL/6 mice; age-related hearing loss in C57BL/6 mice	Activating PTEN-PI3K-Akt axis; inhibiting oxidative stress, apoptosis and inflammation	71–75
		Epicatechin	Radiation-induced ototoxicity in zebrafish and SD rats; cisplatin-induced ototoxicity in SD rats	Inhibiting oxidative stress and apoptosis	76,77
		EGCG	Cisplatin-induced ototoxicity in Wistar rats; amikacin and gentamicin-induced hearing loss in zebrafish	Reducing ROS and inhibiting ERK1/2 and STAT1; inhibiting apoptosis	78,79
		Flavonoids	Galangin	Amikacin-induced ototoxicity in ICR mouse cochlear explants	Inhibiting oxidative stress
Luteolin	Hydrogen peroxide-induced cellular senescence model in HEI-OC1		Promoting sirtuin 1 expression and inhibiting oxidative stress	83	

		Hesperetin	Cisplatin-induced ototoxicity in Wistar rats	Increasing antioxidant enzymes and reducing oxidant parameters	84
		Puerarin	Noise-induced hearing loss in C57 mice; gentamicin-induced ototoxicity in C57BL/6J mice	Regulating the expression of PKC $\gamma$ and GABABR; reducing ROS and inhibiting mitochondria-dependent apoptosis	85–87
	Terpenoids	Ginkgolide B	Cisplatin-induced ototoxicity in SD rats	Reducing ROS and inhibiting mitochondrial apoptosis	88
		Oleuropein	Noise-induced hearing loss in Wistar rats	Reducing ABR hearing threshold shifts and attenuating SGN damage	89
		Oridonin	Noise-induced hearing loss in C57 mice; kanamycin-induced hearing loss in Kunming mice	Inhibiting NLRP3-inflammasome activation and caspase-1/GSDMD-related hair cell pyroptosis	90,91
	Saccharides	LBP	Cisplatin-induced ototoxicity in SD rats	Reducing ROS and maintaining mitochondrial potential	92,93
Extracts of single CHM		<i>Salvia miltiorrhiza</i>	Gentamicin and cisplatin-induced ototoxicity in guinea pigs	Decreasing iNOS and caspase-3 expression	94–96
		<i>Ginkgo biloba</i>	Gentamicin-induced ototoxicity in guinea pigs; cisplatin-induced ototoxicity in Wistar rats; noise-induced hearing loss in SD rats	Reducing ROS and NO and inhibiting apoptosis	97–102
		<i>Astragalus membranaceus</i>	Noise-induced hearing loss in guinea pigs	Reducing the change of connexin 26 in stria vascularis	103,104
		<i>Rehmannia glutinosa</i>	Cisplatin-induced ototoxicity in HEI-OC1 cells	Increasing activity of antioxidant enzymes; inhibiting lipid peroxidation and scavenging activities of free radicals	106–108
		<i>Curculigo orchioides</i>	Cisplatin and noise-induced hearing loss in ICR mice	Inhibiting lipid peroxidation and scavenging activities against free radicals	109–111
CHM prescriptions		Erlong Zuoci	Age-related hearing loss in C57 mice; gentamicin-induced ototoxicity in Kunming mice	Inhibiting mitochondrial apoptosis	112–114
		Jian Er	Age-related hearing loss in C57 mice	Inhibiting oxidative stress and mitochondrial apoptosis	119

**Table 2** Effects of CHM against SNHL in clinical trials.

CHM	Research type	Sample	Intervention	control	Observation period	Outcomes	Ref.
<i>Radix Astragali</i>	Cohort study	92	<i>Radix Astragali</i> was administrated intravenously at the dosage of 0.5 mL/kg/day for 10 continuous days	Non-RA treatment	12 weeks	Pure-tone audiometric threshold change	105
Erlong Zuoci	Prospective randomized controlled study	60	Erlong Zuoci were orally administrated 6 g Bid combined with sound therapy for 3 months	Sound therapy for 3 months	3 months	Pure-tone audiometric threshold change, inflammatory factors	115
Wu Ling San	One-arm observational study	86	Supplemented Wu Ling San decoction was orally administrated 1–2 doses/day	Null	Commonly 2 weeks	Symptoms	116,117
	Retrospective observational study	178	Wu Ling San was orally administrated 7.5 g/day	Steroid, diuretic, drug combinations	7 days	Pure-tone audiometric threshold change	118
Jian Er	Prospective randomized controlled study	80	Jian Er decoction was orally administrated 1 dose/day	Masking method	20–30 days	Symptoms	120
Gushen Pian	Prospective randomized controlled study	120	Gushen Pian was orally administrated 5 tablets, Tid	Placebo	4 weeks	Pure-tone audiometric threshold change, symptoms, Safety evaluation	121,122



3.1.2. Polyphenols

Polyphenols refer to a general term of naturally occurring compounds with phenolic hydroxyl structures, which exhibit potent antioxidant properties and can protect against various chronic diseases induced by oxidative stress. Rosmarinic acid, which is a polyphenol and a water-soluble component isolated from the Chinese herb *S. miltiorrhiza*, inhibits cisplatin-induced apoptosis of auditory cells by targeting caspase 1 and suppressing the activation of downstream signal molecules such as BAX/BCL-2/ caspase 3, NF-κB and cytochrome *c*<sup>62</sup>. In noise-induced hearing loss, rosmarinic acid significantly decreased cochlear oxidative stress and ABR threshold after noise exposure by potentiating NRF2–ARE signaling pathway<sup>63,64</sup>. Curcumin is the most known and major biologically active constituent of *C. longa*. A large number of studies have been conducted to determine its anti-inflammatory, anti-oxidant, anti-fungal, anti-cancer, and other activities<sup>65–69</sup>. The otoprotective effects of curcumin have been evaluated in models of drug- and noise-induced ototoxicity. Curcumin could alleviate gentamicin- and sodium salicylates-induced reduction of glutathione and catalase activity, and increased expression of caspase-3 and NF-κB, thus promoting hearing recovery<sup>70</sup>. Resveratrol is a natural polyphenol abundant in a wide range of plants. Amounts of research have demonstrated the protective effects of resveratrol on auditory dysfunction induced by drugs, noise, and aging<sup>71–75</sup>. The mechanisms of the otoprotective effects of resveratrol may be related to the attenuation of oxidative stress, inflammation, and apoptosis. Epicatechin and epigallocatechin-3-gallate (EGCG) are the major active

compounds in green tea polyphenols from *C. sinensis*. The otoprotective effects of epicatechin have been demonstrated *in vivo* and *in vitro* with its anti-oxidative and anti-apoptotic effects<sup>76,77</sup>. EGCG has been shown to ameliorate cisplatin-induced hair cell and ribbon synapse loss through inhibition of ERK1/2 and STAT1 activation<sup>78</sup>. Other mechanisms of otoprotective effects of EGCG are associated with the reduction of aminoglycosides-induced ROS accumulation and hair cell apoptosis by targeting AKT1, DHFR, and MET<sup>79</sup>.

3.1.3. Flavonoids

Flavonoids, which widely exist in nature and belong to the secondary metabolites of plants, are a class of polyphenolic compounds with a basic structural unit of 2-phenylchromone. Flavonoids can be found almost in all foods of vegetable origin and adequate evidence has demonstrated that flavonoids exhibit strong anti-oxidative, anti-inflammatory, and neuroprotective properties<sup>80,81</sup>. Galangin, the most abundant flavonoid found in the rhizomes of *A. officinarum*, has been proven to exert protective effects on aminoglycoside-induced ototoxicity<sup>82</sup>. The otoprotective effects were attributed to the improvement of hair cell damage and inhibition of mitochondrial ROS production. Luteolin is a natural flavonoid abundant in chamomile. It has been reported that luteolin significantly attenuates hydrogen peroxide-induced auditory HEI-OCI cellular senescence by promoting SIRT1 expression. The protective effect of luteolin was abolished when SIRT1 was downregulated, which indicated that SIRT1 might be a potential target of luteolin<sup>83</sup>. Hesperetin is a flavonoid derived

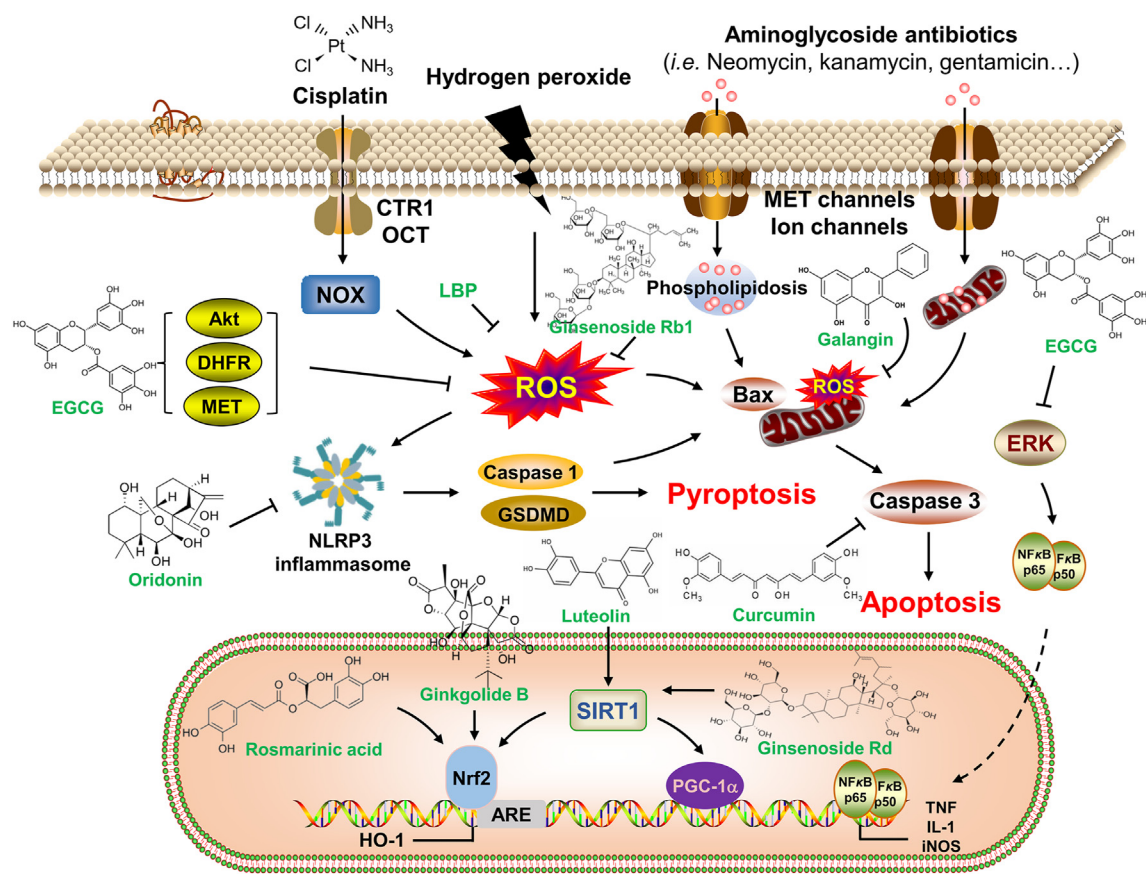


Figure 1 Molecular mechanisms of active ingredients of CHM for the treatment of SNHL.

from *C. aurantium* that acts as an antioxidant. In a cisplatin-induced ototoxicity rat model, it prevented hearing loss at frequencies 8.4, 9.6, and 9.96, and ameliorated cisplatin-induced injury in the organ of corti, spiral ganglion, and stria vascularis<sup>84</sup>. *P. lobata* is traditionally used in China for the treatment of various health ailments<sup>85</sup>. Puerarin is a major isoflavonoid extracted from *P. lobata* and has been investigated for the prevention of hearing loss. Puerarin treatment significantly alleviated the auditory functions in noise-induced hearing loss mice, which might be related to the regulation of the expression of PKC $\gamma$  and GABABR<sup>86</sup>. Puerarin administration also protected against gentamicin-induced cochlear hair cell apoptosis by inhibiting the mitochondria-dependent apoptosis pathway<sup>87</sup>.

### 3.1.4. Terpenoids

Terpenoids, a kind of natural hydrocarbons that widely exist in plants, have important physiological activities and are important sources for studying natural products and developing new drugs. Ginkgolide B is a major terpenoid component of *G. biloba* extract. It has been shown that it can activate the AKT signaling pathway, reduce NOX2 expression, and enhance the NRF2–HO-1 antioxidant pathway, furthermore reducing ROS generation, and inhibiting cisplatin-induced mitochondrial apoptosis *in vivo* and *in vitro*<sup>88</sup>. Oleuropein is a non-toxic secoiridoid glycoside compound isolated from the leaves of olive trees. It has been reported that oleuropein has a partial protective effect against noise-induced hearing loss<sup>89</sup>. The noise-induced elevated ABR thresholds were decreased in the oleuropein group and the damage of spiral ganglion cells was attenuated. Oridonin is a bioactive ent-kaurane diterpenoid isolated from *R. rubescens* of the Labtea family. Treatment with oridonin inhibited the activation of inflammasome in mouse cochleae, protecting against noise-induced hearing loss<sup>90</sup>. And oridonin was also shown to attenuate kanamycin-induced hearing loss by inhibiting NLRP3-inflammasome activation and caspase-1/GSDMD-related hair cell pyroptosis<sup>91</sup>.

### 3.1.5. Saccharides

Saccharides is a general term for polyhydroxy aldehydes or ketones and their derivatives and polymers. Saccharides can be divided into monosaccharides (including glucose, fructose, galactose, etc.), disaccharides (including sucrose, maltose, lactose, etc.), and polysaccharides (including starch, liver glycogen, muscle glycogen, etc.) according to their different structures. Saccharides are the most abundant macromolecules that play a key role in biological processes. *L. barbarum* is a traditional Chinese herbal medicine used as a medicinal and functional food due to its significant beneficial effects. *L. barbarum* polysaccharides (LBP) are the major constituents extracted from *L. barbarum* and have been shown to exert anti-oxidative, immunoregulatory, anti-aging, and neuroprotective properties<sup>92</sup>. It has been reported that LBP pretreatment attenuates cisplatin-induced cochlear hair cell loss. The protective effects of LBP may be attributed to the inhibition of ROS production and maintenance of mitochondrial membrane potential<sup>93</sup>.

## 3.2. Extracts of single CHM

Single CHM extract is a kind of CHM product that is obtained from the extraction, separation, and processing of CHM with advanced technology and has a relatively clear material basis and strict quality standard. Numerous scientific studies of the extracts

of single CHM have demonstrated the *in vitro* and *in vivo* protective effects on SNHL through different target cells and different mechanisms of action (Fig. 2).

### 3.2.1. *Salvia miltiorrhiza*

Rhizome *S. miltiorrhiza*, which is derived from the root of *S. miltiorrhizae* Bunge, has been extensively applied for various diseases for many years and exerts antioxidative, neuroprotective, anti-inflammatory, and antineoplastic activities<sup>94</sup>. It showed that pretreatment with *S. miltiorrhiza* extracts alleviated gentamicin-induced hearing loss by decreasing iNOS and caspase-3 expression, further reducing apoptotic cells<sup>95</sup>. In cisplatin-induced ototoxicity in guinea pigs, *S. miltiorrhiza* attenuated cisplatin-induced significant elevation of ABR threshold and loss of cochlear hair cells. *S. miltiorrhiza* also showed protective effects against cisplatin-induced stria vascularis and spiral ganglion cell injury<sup>96</sup>.

### 3.2.2. *Ginkgo biloba*

As a “living fossil”, *G. biloba* is known as a valuable herb and the extracts of *G. biloba* leaves have been used for medicinal purposes for more than 2000 years in China<sup>97</sup>. *G. biloba* leaves have been among the most widely used CHM for centuries and applied for the treatment of Alzheimer’s disease, stroke, cerebrovascular disability, depression, and vascular dementia<sup>98,99</sup>. By establishing *in vivo* animal models and *in vitro* explant cultures, standardized *G. biloba* leaf extract (EGb761) exhibited a protective effect on gentamicin-induced ototoxicity. EGb761 could significantly decrease gentamicin-induced ROS and NO production and improve auditory function by inhibiting apoptosis of cochlear hair cells<sup>100</sup>. It was reported that *G. biloba* could relieve cisplatin-triggered damage of the Corti organ, spiral ganglion neurons, and striae vascularis<sup>101</sup>. In noise-induced hearing loss, EGb761 could also decrease noise-induced elevated distortion product otoacoustic emission and ABR threshold *via* its antioxidant activity<sup>102</sup>.

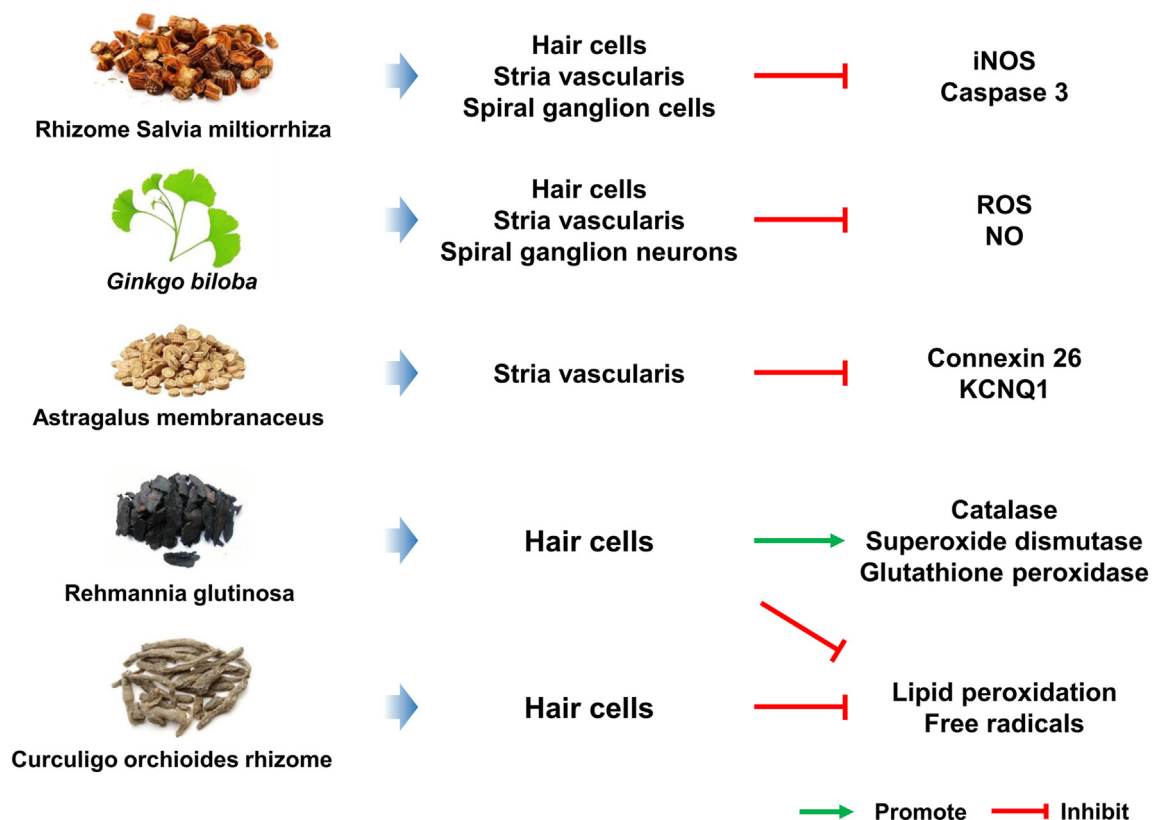
### 3.2.3. *Astragalus membranaceus*

*A. membranaceus* is one of the most significant tonic herbs in CHM and is classified as the qi-invigorating drug in “*Shen Nong Ben Cao Jing*” which is the first book recording CHM in China. It is demonstrated that *A. membranaceus* has prominent immunomodulating, anti-inflammatory, anti-hyperglycemic, anti-oxidant, and anti-viral effects<sup>103</sup>. It has been reported that *A. membranaceus* could obviously attenuate the ABR deficits and stria vascularis damage with the reduction of the expression of connexin 26 and KCNQ1 in stria vascularis after acoustic trauma<sup>104</sup>. In a clinical trial, intravenously administration of *A. membranaceus* in patients with hearing impairment had a better recovery than non-*A. membranaceus* groups<sup>105</sup>.

### 3.2.4. *Rehmannia glutinosa*

*R. glutinosa* belongs to the family of Scrophulariaceae and has multiple pharmacological actions on different systems of the human body such as the immune, nervous, endocrine, and blood system<sup>106</sup>. *In vitro* study showed that the ethanol extract of steamed roots of *R. glutinosa* (SRG) could increase the activity of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase in auditory HEI-OC1 cells, which indicated that SRG had significant anti-oxidant effects<sup>107</sup>. Further research suggested that SRG protected against cisplatin-induced HEI-OC1 cell injury by suppressing lipid peroxidation and





**Figure 2** Illustration of the protective mechanisms of single extracts of CHM for SNHL.

scavenging activities of free radicals<sup>108</sup>. However, there is still limited evidence about the otoprotective effects of *R. glutinosa* and the underlying mechanism of action needs further investigation.

### 3.2.5. *Curculigo orchioides*

*C. orchioides* Gaertn which is widely distributed in China has been considered to have anti-aging, anti-oxidant, anti-cancer, and anti-diabetic activities<sup>109</sup>. The effect of ethanol extract of *C. orchioides* rhizome was evaluated in cisplatin-induced auditory damage *in vivo* and *in vitro*. The result showed that the extract of *C. orchioides* rhizome could attenuate cisplatin-induced cochlear and peripheral auditory function impairments by inhibiting lipid peroxidation and scavenging free radicals<sup>110</sup>. It is also demonstrated that oral administration of the extract of *C. orchioides* reduces noise exposure-induced ABR threshold shifts and cochlear function deficits<sup>111</sup>. These results indicate that *C. orchioides* may be used as a potential therapeutic natural product for SNHL, but the deep molecular mechanisms need further exploration.

## 3.3. CHM prescriptions

### 3.3.1. Erlong Zuoci

Erlong Zuoci Wan (ELZC) which consists of *Radix Rehmanniae Preparata*, *Fructus Corni*, *Rhizoma Dioscoreae*, *Poria cocos*, *Rhizoma Alismatis*, *Cortex moutan*, *Radix Bupleuri* and

*Magnetitum*, is a typical kidney-tonifying CHM prescription available for the treatment of hearing loss. A preclinical study showed that ELZC could decrease the rise of ABR threshold in age-related hearing loss mice. The effects were attributed to attenuating spiral ganglion cell damage by inhibiting the expression level of P53 and BAK<sup>112</sup>. By a combination of network pharmacology with experimental validation, the possible molecular mechanisms underlying ELZC were investigated. The results indicated that the protective effects of ELZC against age-related hearing loss were linked to cellular senescence, inflammatory response, and synaptic connections, and the potential targets and regulatory signal pathways might be associated with AKT, ERK, JNK/STAT3 pathways<sup>113</sup>. Studies also demonstrated that EGZC protected against gentamicin-induced cochlear hair cell injury. Its disassembled prescriptions, Liuwei Dihuang was the main component for the otoprotective effects<sup>114</sup>. A recent clinical trial showed that age-related hearing loss patients treated with ELZC exhibited a better hearing recovery compared with the control group, and there was no serious adverse event after ELZC treatment<sup>115</sup>.

### 3.3.2. Wu Ling San

Wu Ling San (WLS) is a CHM formula that was first recorded in “Shang Han Lun” written by Zhongjing Zhang in the Han dynasty. It is made up of five kinds of Chinese herbs: *Poria cocos*, *Rhizoma Alismatis*, *P. umbellatus*, *C. ramulus*, and *Rhizoma Atractylodis Macrocephalae*. WLS is commonly used for the treatment of

urinary difficulties due to its diuretic properties<sup>116</sup>. WLS has been shown to be effective for Ménière's disease<sup>117</sup>. It was also reported that steroid-WLS combination therapy was more effective than the diuretic alone, WLS alone, or steroid-diuretic combination, indicating that WLS played a critical role in the treatment of acute low-tone sensorineural hearing loss<sup>118</sup>.

### 3.3.3. Jian Er

The CHM prescription Jian Er preparation (JEP) is composed of *Radix astragali*, *Radix puerariae*, *Radix salviae miltiorrhizae*, and *Rhizoma drynariae*. JEP exhibits multifunctional properties such as reduction of blood lipids, prevention of arteriosclerosis, improvement of microcirculation, and reduction of oxidative stress. In the age-related hearing loss model, JEP was shown to decrease the cochlear and auditory cortex malondialdehyde content, lessen mitochondrial DNA damage, and reduce the expression of apoptosis-related protein caspase 3 in cochlear cells, thus protecting against presbycusis<sup>119</sup>. JEP which has been used for the treatment of patients with SNHL also has a good clinical ability to protect hearing in humans<sup>120</sup>.

### 3.3.4. Gushen Pian

Gushen Pian (GSP) which includes *Rhizoma Drynariae*, Calcined *Ci Shi*, *S. miltiorrhiza*, and *G. glabra*, is a pure prescription of CHM. With the function of nourishing kidney, tonifying spleen, resolving phlegm, eliminating dampness, and removing blood stasis<sup>121</sup>, it is effective in the treatment of SNHL and tinnitus due to splenonephric hypofunction and phlegm-accumulation stasis. It has been reported that the GSP has significant therapeutic effects on SNHL patients according to a Phase II clinical trial. It can also attenuate dizziness, insomnia, and fatigue without any adverse effects and toxicity<sup>122</sup>. However, the mechanism of action of GSP on auditory protection should be further investigated.

## 4. Conclusions

There is a very long history of CHM for the treatment of hearing loss in China. In recent years, CHM has made considerable progress in the prevention and treatment of SNHL, which provides adequate experimental evidence for the clinical application of CHM. However, there are still many problems required to be further investigated. For example, the technical indicators are limited to the exploration of morphology and auditory function. Repairment or regeneration of cochlear sensory hair cells are the major biological approaches for restoring hearing function in SNHL, which should also be paid more attention to in the mechanism research of CHM. In this review, the potential molecular mechanisms of CHM for its protection of SNHL we elucidate involve the alleviation of ROS production, inflammation, apoptosis, and pyroptosis in different structures of the auditory system, but there is still limited evidence in the specific pharmacological mechanism of some of the CHM herbs or isolated ingredients.

Nowadays advanced chemical analyses are employed for the separation of various bioactive molecules from the herbs. After being synthetically modified the bioactive compounds exert stronger potentials for the treatment of SNHL and are likely to be further investigated as promising agents. With the development of experimental technologies such as metabolomics, transcriptomics, and proteomics, integrative platforms are set up to give a comprehensive interpretation of CHM mechanisms from a modern biological and medical view. Therefore, it is urgent to find safe and effective therapies *via* the development of CHM with novel

mechanisms of action, and extensive clinical trials will be required to find the most effective treatments. With the development of new technologies and methods, we expect to see exciting breakthroughs in the near future.

## Acknowledgments

This work was supported by grants from the National Key R&D Program of China (Nos. 2021YFA1101300, 2021YFA1101800, and 2020YFA0112503), Strategic Priority Research Program of the Chinese Academy of Science (XDA16010303, China), National Natural Science Foundation of China (Nos. 82101228, 82030029, 81970882, and 92149304), Science and Technology Department of Sichuan Province (No. 2021YFS0371, China), Shenzhen Science and Technology Program (JCYJ20190814093401920 and JCYJ20210324125608022, China), and Open Research Fund of State Key Laboratory of Genetic Engineering, Fudan University (No. SKLGE-2104, China).

## Author contributions

Yunhao Wu, Wenyong Chen, and Renjie Chai conceived the manuscript. Yunhao Wu, and Jingwen Zhang wrote the manuscript. Qiuping Liu and Zhuang Miao gave advice and suggestions.

## Conflicts of interest

The authors declare no competing financial interests.

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