Mitochondrial MPTP: A Novel Target of Ethnomedicine for Stroke Treatment by Apoptosis Inhibition

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Mammalian mitochondrial permeability transition pore (MPTP), across the inner and outer membranes of mitochondria, is a nonspecific channel for signal transduction or material transfer between mitochondrial matrix and cytoplasm such as maintenance of Ca²⁺ homeostasis, regulation of oxidative stress signals, and protein translocation evoked by some of stimuli. Continuous MPTP opening has been proved to stimulate neuronal apoptosis in ischemic stroke. Meanwhile, inhibition of MPTP overopening-induced apoptosis has shown excellent efficacy in the treatment of ischemic stroke. Among of which, the potential molecular mechanisms of drug therapy for stroke has also been gradually revealed by researchers. The characteristics of multi-components or multi-targets for ethnic drugs also provide the possibility to treat stroke from the perspective of mitochondrial MPTP. The advantages mentioned above make it necessary for us to explore and clarify the new perspective of ethnic medicine in treating stroke and to determine the specific molecular mechanisms through advanced technologies as much as possible. In this review, we attempt to uncover the relationship between abnormal MPTP opening and neuronal apoptosis in ischemic stroke. We further summarized currently authorized drugs, ethnic medicine prescriptions, herbs, and identified monomer compounds for inhibition of MPTP overopening-induced ischemic neuron apoptosis. Finally, we strive to provide a new perspective and enlightenment for ethnic medicine in the prevention and treatment of stroke by inhibition of MPTP overopening-induced neuronal apoptosis.

Keywords: ischemic stroke, mammalian mitochondrial permeability transition pore, mitochondrial apoptosis, ethnic medicine, prescription, monomer composition

INTRODUCTION

The mitochondrial permeability transition pore (MPTP) complex is a non-specific and -selective channel composed of multiple proteins, which is voltage-dependent and spans cytoplasm, outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), and mitochondrial matrix. Excessive MPTP opening has been reported in relation to myocardial ischemia reperfusion...
injury (Morciano et al., 2017), hepatic ischemia-reperfusion injury (Panel et al., 2019), traumatic brain injury (Hänell et al., 2015), premature aging (Zhou et al., 2019), and Parkinson’s disease (Ludtmann et al., 2018). However, its structural composition of MPTP (Baines and Gutiérrez-Aguilar, 2018) and detailed regulatory mechanism in ischemic stroke are still poorly understood. To our knowledge, current evidences support the fact that MPTP is composed of voltage-dependent anion channel (VDAC) across the OMM, adenine nucleotide translocator (ANT) in the IMM, and cyclophilin D (CypD) in the mitochondrial matrix, which is responsible for sensing intracellular environmental oxidative stress injury, inflammatory cascade, pH imbalance, and ion disorders such as Ca\(^{2+}\) and Mg\(^{2+}\) ions in response to tissue ischemia (Kalani et al., 2018; Briston et al., 2019). These adverse factors, alone or together, can force persistent and irreversible MPTP opening beyond the range of physiological regulation, and thus inducing mitochondria-dependent apoptotic events. In addition, cytoplasmic hexokinase II (HK II) attached to VDAC, the peripheral benzodiazepine receptor (PBR) on OMM and creatine kinase responsible for ATP production may be involved in the formation or regulation of MPTP (Zamzami and Kroemer, 2001). Possibly as a component of IMM and binding partner of CypD, the phosphate carrier (PiC) of mitochondria is responsible for the supply of inorganic phosphates required by ATP synthesis during oxidative phosphorylation of mitochondria (Brenner and Moulin, 2012; Bernardi et al., 2015; Solesio et al., 2016). However, whether PiC has a positive or negative effect on the structure and function of MPTP, it is still a matter of debate and disagreement. Figure 1 illustrates the canonical molecular composition of MPTP.

In recent years, several other members involved in MPTP regulation responsible for cell fate decision have also been identified in succession. As one of the core components of IMM, RNAi-targeted silencing of the spastic paraplegia 7 gene blocked signal transmission between OMM and mitochondrial matrix by indirectly associating VDAC with CypD in the matrix, thereby abrogating overloaded Ca\(^{2+}\) and immoderate ROS evoked mitochondrial membrane potential (MMP) decline and MPTP-dependent cell death (Shammughapriya et al., 2015). Except for the re-confirmation that ANT was the basic intimal component of MPTP, researchers had also found that other CypD-dependent components were involved in the composition of MPTP. Although the species destined for existence had not yet been identified, the authors suggested that PiC such as the Slc25a3 or F_{1}F_{0} ATP synthase may be involved, which still needed to be explored in a reasonable and rigorous in vivo and in vitro experiment (Karch et al., 2019). Further encouraging evidence suggested that F-ATP synthase was involved in the formation of MPTP, sensing Ca\(^{2+}\) concentration and subsequently mediating MPTP opening (Urbani et al., 2019).

As the structure and functions of MPTP are gradually clarified, great quantities of studies have declared that abnormal MPTP conditions play a critical role in regulating cell fate in a variety of diseases. The VCAD has virtually no barrier effect on small molecules with molecular weights less than 5 kDa to circulate freely in the cytoplasm and mitochondrial matrix (Bonora and Pinton, 2019). As an intermediate bridge, ANT can interact directly with VCAD and CypD. And that is, ANT can alter OMM and IMM permeability by regulating VCAD and CypD, thus mediating the exchange of substances in the cytoplasmic matrix and the mitochondrial matrix (Chinopoulos, 2018). In a mouse model of heart failure, it had been substantiated that increased mitochondrial matrix Ca\(^{2+}\) caused by Ppif gene (encoding the synthesis of CypD protein) deficiency contributed to the remission of heart failure symptoms (Elrod et al., 2010). By further silencing CypD gene with in vitro siRNA and shRNA techniques on primary human pulmonary artery endothelial cells, and in vivo CypD knockout mice, evidence of CypD deficiency had been shown to promote angiogenesis, which may be partly due to increased mitochondrial matrix Ca\(^{2+}\) and nicotinamide adenine dinucleotide (NADH), activation of NAD\(^{+}\)-dependent deacetylase sirtuin 1 (SIRT1) and serine-threonine kinase Akt signaling (Marcu et al., 2015). Evidence suggested that induced pluripotent stem cells (iPSCs) derived hepatocyte toxicity caused by valproic acid was associated with MPTP opening dependent mitochondrial apoptotic pathway (Li et al., 2015).

**Causality Between Abnormal MPTP Opening and Apoptosis in Ischemic Stroke**

Abnormalities of MPTP state are bound to trigger cellular dysfunction in ischemic stroke. We will briefly summarize the factors and related molecular mechanisms of MPTP opening-induced apoptosis after ischemic stroke. A large number of previous reports have shown that stroke-evoked decreased MMP, excessive mitochondrial reactive oxygen species (mtROS) (Zorov et al., 2014), endoplasmic reticulum stress (ERS), and excitatory amino acid toxicity all stimulated MPTP opening (Prentice et al., 2017).
2015), leading to mitochondrial edema, increased membrane permeability, corrupted cristae structure of IMM, and neuronal apoptosis. Notably, as the second messenger, Ca$^{2+}$ is a stimulus of MPTP opening and also could be a landmark event after MPTP opening. However, from the actual effect, increased Ca$^{2+}$ and depressed matrix Mg$^{2+}$ and Mn$^{2+}$ could all contribute to MPTP opening. In turn, evidence had announced that instantaneously MPTP opening could cause increased Ca$^{2+}$ in microdomain of astrocytes, which was closely related to maintaining mitochondrial energy supply and stress response (Agarwal et al., 2017). The otherwise MPTP opening-prone factors are as following. Declined matrix PH, caused by protonation of histidine residues or loss of ANT and CypD signaling, could trigger MPTP to tend to shut down. Conversely, the increased matrix PH forces MPTP opening with its maximum openness at about 7.3 (Wang et al., 2016; Šileikytė and Forte, 2019). The formation of disulfide by oxidation on ANT dimer, oxidized pyridine nucleotides such as NAD$^+$ and NADP$^+$ all favor MPTP openness. Conversely, all the factors that inhibit MPTP opening may have a promising future in treating ischemic stroke. Ligands targeting VADC, ANT, CypD (Matsumoto et al., 1999), and TSPO/PBR targets have shown better inhibition of MPTP opening. Moreover, antioxidants such as propofol, metabolites such as glucose and creatine, coenzyme Q, glutamate, or Ca$^{2+}$ chelators could limit MPTP opening (Zamzami and Kroemer, 2001; Brenner and Moulin, 2012).

It is well known that onset of ischemic stroke causes neurons to produce exorbitant mtROS, ERS, Ca$^{2+}$ overload, and neuronal toxicity induced by excitatory amino acids. After that, neurons would raise the alarm of MMP decline, mitochondrial edema, elevated MMP and other signs of MPTP opening, which will eventually drive mitochondrial contents such as Cyto-c to be discharged into the cytoplasm and trigger apoptotic events. The results of in vivo animal evaluation have intimated that both transient and permanent cerebral ischemic insults can cause damage to mitochondrial ultrastructure of neuron, such as the appearance of swollen and condensate mitochondria, as well elevated matrix density caused by deposition of electron-dense material (Solenski et al., 2002). An ischemia-induced ROS elevation can favor MPTP opening, which in turn can lead to a subsequent surge in ROS production and a vicious cycle (Zorov et al., 2014). Therefore, inhibition of neuronal apoptosis by blocking MPTP opening would be a potential and promising strategy in the treatment of ischemic stroke. Further extensive in vivo and in vitro experimental evidence also suggested a positive effect of this therapy. In rat models of ischemic stroke, blocking MPTP opening by cyclosporine A had been shown to reduce infarcted volume of ischemic brain tissue (Matsumoto et al., 1999). As a ligand targeting CypD, pre-administration of cyclosporine A can protect primary rat neurons from OGD/R injury, involved mechanisms may be related to maintain mitochondrial integrity and inhibit MPTP opening-induced apoptosis by up-regulating Parkinson’s disease-associated protein DJ-1 (Tajiri et al., 2016). Further, the water-soluble coenzyme Q10 had been shown to protect the accumulation of glutamate-induced HT22 hippocampal neuron damage by inhibiting mitochondrial fragmentation and MPTP opening-induced apoptosis (Kumari et al., 2016). Furthermore, evidence had shown that intervention of MPTP opening inhibitor can reduce the expression of VDAC, manifesting by increased MMP, ATP supply, and improved cerebral ischemia injury symptoms in an in vitro rat model of MCAO (Wang et al., 2019a). The above evidence all conveys that ischemic stroke induced MPTP opening may be a factoid of neuronal apoptosis. Any measures to inhibit MPTP opening could repress cell apoptosis, thus exhibiting the role of anti-ischemic brain protection.

Explosive evidence corroborated that a sudden insult of ischemic stroke may break the balance between the anti-apoptotic and pro-apoptotic members of B-cell lymphoma-2

![Canonical mitochondrial MPTP molecular structure. Conventional MPTP complex is composed of VDAC, ANT, and CypD. Other factors could also stimulate MPTP opening.](image-url)
decreased neuronal apoptosis were related to the decreased in vitro expression (Mattiasson et al., 2003; Mehta and Li, 2009). The ischemic stroke was mitochondrial uncoupling protein 2 (UCP-2), a potential target involved in regulating mitochondrial MPTP in activation of neuron mitochondrial cannabinoid receptor 1 macropores (Vaseva et al., 2012). It has been reported that apoptosis, which is independent of the formation of Bak/Bax macropores (Seervi et al., 2018). Therefore, the increased activation of Caspase-3/-9, PARP, and overexpressed hyperchromatic nucleus, loss of MMP, reduced Bcl-2, Bak/Bax-dependent apoptosis, showing condensed and increased permeability caused by the formed Bax/Bak dimer on OMM contributed to the release or transfer of pro-apoptotic Cyto-c, Smac/Diablo and HtrA2/Omi from the mitochondrial matrix to cytoplasm (Arnoult et al., 2003). Further rat models of focal cerebral ischemia also demonstrated that overexpressed Bcl-2 protein could inhibit the rise of Cyto-c in cytoplasm, thereby preventing the occurrence of apoptotic DNA fragmentation events mediated by the transfer of AIF from mitochondria to nucleus (Zhao et al., 2004). While pro-apoptotic Bcl-xL-induced apoptosis via Bak also induced the exudation of mitochondrial Cyto-c, the formation of apoptosome composed of Cyto-c, Apaf-1 and Caspase-9, and the Caspase apoptotic cascade (Lindenboim et al., 2005; Zhang Q. et al., 2019). Visually breaking news, Bak/Bax macroprobes contribute to the outflow of mitochondrial contents such as Cyto-c and mitochondrial DNA into the cytoplasm, and thereafter inducing caspase-dependent cell apoptosis (McArthur et al., 2018). Amusingly, evidence also suggested that ROS and ERS could directly activate Bak/Bax-dependent apoptosis, showing condensed and hyperchromatic nucleus, loss of MMP, reduced Bcl-2, increased activation of Caspase-3/-9, PARP, and overexpressed Bak and Bax proteins (Seervi et al., 2018). Therefore, the formation of Bak/Bax macroprobes in mitochondrial OMM may serve as a hub for MPTP opening-induced mitochondrial apoptosis. Other factors involved in the regulation of MPTP opening after cerebral ischemia have also been reported. Accumulation of p53 in mitochondria has been corroborated to target CypD, leading to MPTP opening and neuronal apoptosis, which is independent of the formation of Bak/Bax macroprobes (Vaseva et al., 2012). It has been reported that activation of neuron mitochondrial cannabinoid receptor 1 after cerebral ischemia can help inhibit Ca2+ overload-induced MPTP opening and apoptosis (Cai et al., 2017). Another potential target involved in regulating mitochondrial MPTP in ischemic stroke was mitochondrial uncoupling protein 2 (UCP-2). Highly expressed UCP-2 has been demonstrated to inhibit apoptosis by activating redox signaling, evidenced by decreased ROS production, increased MMP and cleaved Caspase-3 protein expression (Mattiasson et al., 2003; Mehta and Li, 2009). The above analysis indicates that ischemic stroke is accompanied by an inevitable event of MPTP over-opening and apoptosis. Although the basic structure of MPTP has not yet been drastically uncovered and recognized. But a number of factors that regulate MPTP opening during the course of ischemic stroke have been exposed in the public eye. In the future, plenty of basic studies should be conducted to elucidate the molecular composition of MPTP and its relationship with ischemic neuron apoptosis. Meanwhile, natural product inhibitors targeting MPTP opening-evoked neuronal apoptosis are also worthy of further research in the treatment of ischemic stroke.

**PROGRESS IN STROKE PREVENTION AND TREATMENT BY REGULATING MITOCHONDRIAL MPTP STATUS IN ETHNIC MEDICINE**

Ischemic stroke, which accounts for 71% of stroke, is the second and the first leading cause of death and disability worldwide and in China, respectively (Wu S. et al., 2019). In 2016, there were 9.5 million ischemic stroke patients worldwide, while in 2017, 2.7 million people died of ischemic stroke (Campbell et al., 2019). Although intravenous thrombolysis, antiplatelet aggregation, and anticoagulant therapy (Smith et al., 2019; Stoll and Nieswandt, 2019) could be used for the delivery of stroke therapies, but many apoplexy sequela, characterized by ischemic contralateral or bilateral limb behavior disorders, memory decay, logopathy, dysphagia, and mood irritability (Zhao et al., 2016; Hou et al., 2020), have not yet cure. However, the ethnic medicine has manifested significant clinical efficacy in alleviating above unbearable symptoms or sequelae of stroke. In recent years, the mechanisms of action of drugs have also been gradually revealed. Remarkably, some of them, such as Danhong injection and Naoxintong capsule (Haiyu et al., 2016; Liu M. et al., 2016; Xu et al., 2020), have been officially approved by the China Food and Drug Administration (CFDA) and are bringing good news to stroke patients around the world. Next, we summarized the current officially authorized products, clinically effective traditional Chinese medicine (TCM) prescriptions, ethnic drugs, and effective monomer components based on literature review, trying to clarify the molecular mechanisms of natural products inhibiting neuronal apoptosis and improving ischemic brain from the perspective of mitochondrial MPTP.

**Authorized Products for Stroke Improvement by Regulating Mitochondrial MPTP**

With the policy guidance and inclination, as well as the accelerated modernization of TCM, tens of thousands of individuals dedicated to clinical and scientific research positions are gradually devoting themselves to the drug development and mechanism exploration of traditional medicine to prevent major diseases, such as stroke. Most
ethnic drugs for treatment of ischemic stroke have the function of activating blood circulation to remove blood stasis or clear collaterals. NaoShuanTong capsule (Zhang H. et al., 2019), ShenQi Fuzheng injection (Cai et al., 2016), ShengMai injection (Yang et al., 2016), and PeiYuan TongNao capsule (Bai J. et al., 2019) have been reported to significantly improve the symptoms of ischemic stroke with few adverse events. In recent years, some antiapoptotic protective effects of cerebral ischemia have also been reported, such as XueShuanTong injection (Li et al., 2009) and QianCao NaoMaiTong mixture (Lu et al., 2016).

Most, such as Cerebralcare Granule® (Sun et al., 2010), DanHong injection (Xu J. et al., 2017; Li M. et al., 2018), and AnGong NiuHuang wan (Wang G. et al., 2014; Tsoi et al., 2019), can inhibit ischemia-evoked neuronal apoptosis by regulating bcl-2 family members. As a prescription commonly used in Tibetan medicine to treat ischemic sequelae, our research group proved that the anti-cerebral ischemia effect of ErShiWei ChenXiang pills may be related to its regulation of Bcl-2 family, inhibition of apoptosis, and increase of energy supply (Hou et al., 2020). While regulating Bcl-2 family members, AnNao tablets (Zhang et al., 2020) and YiQi FuMai powder injection (Cao et al., 2016; Xu Y. et al., 2017) may also be involved in inflammation and mitochondrial autophagy to maintain mitochondrial MMP and energy production. In addition, both TongXinLuO’s regulation of AKT/ERK signaling (Yu et al., 2016; Cheng et al., 2017) and XingNaoJing injection’s regulation of the PI3K-AKT pathway (Zhang Y. et al., 2018) ultimately contributed to the regulation of Bcl-2 and the inhibition of ischemic neuron apoptosis. In addition to the Bcl-2 family, it was reported that Zhenlong Xingnao capsule (Wei X. et al., 2019) and NaoLuoTong capsule (Bai M. et al., 2019) could also be through the regulation of NF-κB to confine ischemia induced inflammatory cascade process. Of course, multiple mechanisms of drugs have also been reported against ischemic neuron apoptosis. QingKaiLing injection could simultaneously inhibit oxidative stress, activation of NLRP3 inflammosome and AMPK signaling pathway, and thus inhibiting neuronal apoptosis (Cheng et al., 2012). PienTzeHuang capsule suppressed the inflammatory and apoptotic cascade of ischemia by regulating AKT/GSK-3β and the Bcl-2 family (Zhang X. et al., 2018). As a fatal blow to the body, disregardful ischemic stroke induced hypoinnununity was also one of the main culprits of exacerbating stroke. Noteworthy, XueSaiTong (Li et al., 2019) and Danggui-Jakyak-San (Kim et al., 2016) may mediate inflammatory responses by regulating STAT3 signaling pathway, and enhance immune function of the body, which were helpful to reduce symptoms of brain injury after ischemia. The above officially certified drugs’ information and specific mechanisms of action are shown in Supplementary Table 1, and Tables 1 and 2. Through in-depth comparative analysis, we found that although the above drugs prevailing in the market have good clinical efficacy, most of their active ingredients, in vivo pharmacokinetic parameters, and potential targeted organ toxicity have not been well evaluated. Importantly, the further regulation of apoptosis still has good research value and prospect. Although there is no direct evidence that they regulate MPTP to inhibit ischemic neuron apoptosis, their effect on members of the Bcl-2 family makes MPTP a potential target for anti-stroke drugs.

**Prescription and Molecular Mechanisms in Regulating MPTP Openness of Ischemic Stroke**

Clinical experience has proved that TCM has excellent efficacy in treating stroke, which can be seen in Huangdi Neijing. But at bottom it is the cold, hot, warm, cool, and other characteristics of drugs to balance the imbalance of Yin and Yang in the body under the condition of disease. In ischemic stroke, a variety of exogenous pathogens and dysfunction of the viscera can lead to poor blood flow or blood stasis, resulting in cerebral ischemia or hypoxia (Hou et al., 2020). Therefore, the clinic mainly focuses on promoting blood circulation to remove stasis, replenishing Qi to nourish blood, and nursing viscera. Extensive clinical and in vivo and in vitro studies have confirmed that prescriptions SiJunZi decoction (Yang et al., 2019), ShengMai san (Li et al., 2013), and YangYin TongNao granules (Wang et al., 2019) have a significant effect on ischemic stroke. Of course, the regulation of oxidative stress and inflammatory response are also common mechanisms of prescription in the treatment of ischemic brain injury. The antioxidant and anti-inflammatory activities of ShengNaoKang decoction (Chen et al., 2014) could contribute to the inhibition of apoptosis and the alleviation of ischemic brain injury. Other studies have reported that HuangLian JieDu decoction (HJ) could inhibit ischemic neuron apoptosis by regulating PI3K/AKT and HIF-1α/VEGF (Zhang Q. et al., 2014). Further metabolomics (Zhu et al., 2018) and systemic pharmacology (Wang P. et al., 2019) studies have revealed that its anti-ischemic protective effect may also involve the Bcl-2 family such as Bak. Regulating vascular function and increasing cerebral blood flow supply is another effective strategy for stroke treatment. Abundant evidence demonstrated that BuYang HuanWu decoction (BHD) could increase cerebral blood by regulating HIF-1α/VEGF-related signaling pathways (Chen et al., 2019). Improving the mitochondrial ATP supply has also been shown to be an effective treatment for stroke. BHD has been reported to improve ischemic brain injury by reducing glutamate-mediated excitatory amino acid toxicity, resulting in enhanced ATP supply and weakened apoptosis (Wang et al., 2011). At the same time, the improved synaptic ultrastructure by BHD also contributed to the recovery of cerebral ischemia sequelae (Pan et al., 2017). Similarly, ShenGui SanSheng san could also improve the efficiency of citric acid cycle to improve the brain energy deficit after ischemia (Luo et al., 2019). Interestingly, as a cell-sensing oxygen sensor, most studies have also reported evidence of other TCM prescriptions regulating HIF-1α to inhibit apoptosis and inflammation in treatment of stroke, such as XueFu ZhuYu decoction (Lee et al., 2011) and TaoHong SiWu decoction (Yen et al., 2014). Members of the Bcl-2 family are also potential targets for prescription inhibition of apoptosis to improve ischemic brain injury. XiaoXuMing decoction (Lan et al., 2014), ShuanTongLing
| Agents | Objects | Gender | Weight (g) | Animal model | Dose | Time periods | Mechanisms | References |
|--------|---------|--------|------------|--------------|------|--------------|------------|------------|
| XueShuanTong injection | SD | Both | 270–320 | MCAO (2 h) | 25 mg/kg, i.p. | Pretreatment for 5 min and 12/24/36 h after MCAO | Caspase-1/3↓, TUNEL-positive neurons↓ | Li et al., 2009 |
| | | | | R (48 h) | | | | |
| Cerebralcare granule | Mongolian gerbils | Male | 65–90 | MCAO (0.5 h)/(5 d) | 0.4 and 0.8 g/kg, i.g. | 3 h after the reperfusion, 5 d, q.d. | Bcl-2↑; leukocyte adhesion↓, fluorescence intensity of DHR↓, albumin leakage↓, | Sun et al., 2010 |
| AnGong | SD | Male | 250–280 | MCAO (1.5 h)/(21/24 h) | 0.065, 0.125, and 0.25 g/kg, i.g. | Pretreatment 3 d, q.d., and 1 d, q.d. after reperfusion | Caspase-3↓, Bax↓, TUNEL-positive neurons↓ | Wang G. et al., 2014 |
| NiHuHuang wan. | SD | Male | 260–280 | MCAO (2 h)/R (22 h) | 287 mg/kg, i.g. | Single dose before reperfusion | Bcl-2↑, TUNEL-positive neurons↓ | Li et al., 2010 |
| | | | | | | | | |
| QingKaiLing injection | KM/CSBL/6 | Male | 25–28/20 | MCAO (1.5 h)/(22 h) | 3 ml/kg, i.v. | 4 h after reperfusion, and once every 12 h, three times | Caspase-1/3↓, TUNEL-positive neurons↓ | Tsoi et al., 2019 |
| DangGui | SD | Male | 65 | pMCAO (28 h) | 50, 100, and 200 mg/kg, i.g. | Pretreatment for 24 h after surgery, 28 d, q.d. | STAT3↓, Bcl-2↑; Bax↓, p47phox↓, ZO-1↓, claudin-5↓, eNOS↑, | Cheng et al., 2012 |
| Jakyak san | SD | Male | 260 | pMCAO (24 h) | 1.342 g/kg, i.p. | Single dose after pMCAO onset | Bcl-2↓, TUNEL-positive neurons↓ | Kim et al., 2016 |
| YiQi FuMai powder injection | SD | Male | 280 | MCAO (2 h) | 0.957 g/kg, i.p. | Single dose after tMCAO onset | Bcl-2↑, TUNEL-positive neurons↓ | Cao et al., 2016 |
| TongXinLuo | SD | Male | 240 | MCAO (1.5 h)/(21/24 h) | 4 ml/kg, i.p. | 4 h after reperfusion, and after MCAO for 1 d, b.i.d. | Procaspase-12↑; Caspase-3↑, p-eIF2α↑, ROS↓, Ca2+↑, TUNEL-positive neurons↓ | Wei X. et al., 2019 |
| QinCao | SD | Male | 18–22 | pMCAO (24 h) | 1.342 g/kg, i.p. | Pretreatment for 5 d and 14 d after MCAO, q.d. | Connexin 43↑, Calpain II↑, Bax↓, cleaved Caspase-3↓, TUNEL-positive neurons↓ | Hou et al., 2019 |
| NaoMaTong mixture | SD | Male | 280–300 | MCAO (1.5 h)/(24 h) | 0.4, 0.8, and 1.6 g/kg, i.g. | Pretreatment for 3 d, b.i.d., and after MCAO for 1 d, b.i.d. | p-PTEN/PTEN↑, p-AKT/308/473/AKT↑, p-PI3K/PI3K↑, p-PDK1/PDK1↑, cleaved Caspase-3↓, TUNEL-positive neurons↓ | Yu et al., 2017 |
| DangHai | SD | Male | 250 | MCAO (2 h) | 270/14 d | Pretreatment for 28 d | Connexin 43↑, Calpain II↑, Bax↓, cleaved Caspase-3↓, TUNEL-positive neurons↓ | Zheng X. et al., 2018 |
| NaoLuoTong capsule | Wistar | Male | 250–280 | MCAO (2 h) | 250/14 d | Pretreatment for 7 d, q.d. | NeuroN↓, ntCyto-c↑, Bcl-x↑, Bax↓, p-PI3K/PI3K↑, p-eIF2α↓, p-PI3K/PI3K↑ | Zhang et al., 2018 |
| ErShWei | SD | Male | 250 | MCAO (2 h) | 75, 150, and 300 mg/kg, i.g. | Pretreatment for 7 d, q.d. | NeuroN↓, ntCyto-c↑, Bcl-x↑, Bax↓, p-PI3K/PI3K↑, p-eIF2α↓, p-PI3K/PI3K↑ | Zhang et al., 2018 |
| ChenXiang pills | SD | Male | 250 | MCAO (2 h) | 1.33 and 2.00 g/kg, i.g. | Pretreatment for 14 d, q.d. | T-AOC↑, T-NO↑, TGF-β↑, IL-10↑, IL-1β↑, STAT3↑, CD16↑, CD14/CD11b↑, TUNEL-positive neurons↓ | Wei X. et al., 2019 |
| AnNao tablets | SD | Male | 250 | MCAO (2 h) | 1.33 and 2.00 g/kg, i.g. | Pretreatment for 14 d, q.d. | T-AOC↑, T-NO↑, TGF-β↑, IL-10↑, IL-1β↑, STAT3↑, CD16↑, CD14/CD11b↑, TUNEL-positive neurons↓ | Wei X. et al., 2019 |

↑ upgrade; ↓ downgrade.
TABLE 2 | The in vitro mechanism underlying the inhibition of MPTP opening-induced neuronal apoptosis by authorized drugs in the treatment of ischemic stroke.

| Agents             | Cell lines      | Model                      | Dose    | Time periods             | Mechanisms                                                                 | References          |
|--------------------|-----------------|----------------------------|---------|--------------------------|-----------------------------------------------------------------------------|---------------------|
| TongLuo JuNao      | BMECs of SD rats | OGD (95% N₂ and 5% CO₂ 6 h/R) | 2 µl/ml  | Before OGD, the neurons were incubated 6 h in drug treatment and then equilibrated OGD | VEGF↑, MMP↑; LDH↓, Ca²⁺↑, cytosolic Cyto-c↓, NMDAR1↓, PAF↓                  | Li et al., 2014     |
| NaoMaiTong mixture | SH-SYSY         | OGD (N₂, 1 h)/R              | 0.5, 1, 5, 10, 50, 100 and 200 µg/ml | Pretreatment for 2 h and during reperfusion period | Caspase-3/8↓, neuronal apoptosis under flow cytometry↓                       | Lu et al., 2016     |
| YiQi FuMai powder  | PC12            | OGD (5% CO₂, 94% N₂, and 1% O₂, 12 h) | 100 mg/ml | during OGD period | Bcl-2↑; neuronal apoptosis under flow cytometry↓, Caspase-3↓, Cleaved Caspase-3↓, Caspase-12↑, CHOP↑, GADD153↑, ATP↓, ADP↓, eIF2α/eIF2α↓, XBP-1↑, Hoochest 33342 positive neurons↑ | Cao et al., 2016    |
| injection          | PCN of embryonic, 16-18-d SD rats | 100 µM H₂O₂ for 12 h | 100, 200, and 400 µg/ml | 6 h before and during H₂O₂ treatment | ATP↑, MMP↑, Bcl-2↑, Bcl-xl↑, cytosolic Drp1↓, cytosolic PKC↓, mROS↓, PKC↓, neuronal apoptosis under flow cytometry↑, intracellular ROS↓, p-Drp1/Drp1↓, mtDrp1↑, mtPKC↓↑ | Xu Y. et al., 2017  |
| injection          | PCN of embryonic, 14-d C57 BL/6 mice | OGD (95% N₂, and 5% CO₂, 6 h) | 0.01, 0.03, 0.1, 0.3, and 1 µl/ml | During OGD period | LDH↓, ROS↓, Ca²⁺↑, neuronal apoptosis under flow cytometry↓               | Xu J. et al., 2016  |
| injection          | HBMCEs          | OGD (5% CO₂, 85% N₂, and 10% H₂O₂, 3 h/R) | 1.5 and 2.5 µl/ml | Pretreatment for 1 h and during reperfusion period | p-eNOS/eNOS↑, MMP↑, NO↑; cleaved Caspase-3/Caspase-12↑, neuronal apoptosis under flow cytometry↑ | Zhang Y. et al., 2018 |

↑, upregulate; ↓, downregulate.

(Mei et al., 2017), and GuaLou Guizhi decoction (Zhang Y. et al., 2014) all have the potential to regulate the Bcl-2 family and inhibit caspase-dependent mitochondrial apoptosis, which has a similar mechanism to that of MuXiang You fang (Zhao et al., 2016) reported in our previous study. In addition to the Bcl-2 family, DiHuang YinZi (Hu et al., 2009) and DiDang tang (Huang et al., 2018) could also inhibit the generation of Ca²⁺ and improve MMP to inhibit the apoptosis of ischemic neurons by regulating the ERK signaling pathway. It has also been reported that HouShiHei san (Chang J. et al., 2016) could regulate PI3K/Akt signaling to inhibit the apoptosis of ischemic neurons. The specific mechanisms in vivo and in vitro of the above prescriptions are shown in Table 3. The above evidence indicates that most TCM prescriptions could more or less improve mitochondrial morphology and respiratory function by inhibiting neuronal Ca²⁺ overload through anti-oxidative stress and anti-inflammatory. Meanwhile, we note that most of them also regulates many members of the Bcl-2 family to inhibit ischemic neuron apoptosis. We, therefore, see the potential of drugs to indirectly inhibit MPTP opening to improve ischemic neuron apoptosis. Nevertheless, the unclear drug distribution of target organs and the intricate network of interactive targets should still drive us to further study.

Herbal Extracts and Molecular Mechanisms in Regulating MPTP Openness of Ischemic Stroke

The overall concept of TCM and the characteristics of treatment based on syndrome differentiation of ethnic medicine determine that prescriptions from diversified drug sources are mainly used in the treatment of diseases. The purpose is to comprehensively consider the functions of viscera to exorcize evil spirits while strengthening the body, and finally cure diseases. However, in addition to conventional prescriptions mentioned above, people have also discovered that the individual application of certain herbs also has the potential to treat diseases. Based on recent literature reports, most of them exhibit outstanding antioxidant effects, such as methanol extract of Artemisia absinthium (Bora and Sharma, 2010) and Colebrookea oppositifolia Smith (Viswanatha et al., 2018). As the most sensitive hippocampal neuron to ischemic invasion, studies have shown that Moringa oleifera seed extract could promote hippocampal nerve regeneration, enhance synaptic plasticity and cholinergic function to treat ischemic stroke (Zeng et al., 2019). More interestingly, Gynostemma pentaphyllum extract could protect OGD/R-induced rats isolated hippocampal slices damage by inhibiting neuronal Ca²⁺ overload and mitochondrial oxidative stress-induced MPTP opening (Schild et al., 2009), which may help to inhibit the MPTP opening-activated mitochondrial apoptotic cascade event. At the same time, herbs could regulate the expression level of anti-apoptotic and pro-apoptotic proteins of Bcl-2 family and inhibit mitochondrial apoptosis in the treatment of hypoxia brain injury. The specific in vivo and in vitro mechanisms of reported herbs for ischemic stroke treatment by inhibiting mitochondrial MPTP opening-induced neuronal apoptosis are shown in Tables 4 and 5. Figure 2 shows pictures of 16 representative herbs. It is world-renowned that superior immune enhancement of plant polysaccharides could prevent and cure many diseases. Previous investigations reported
the anti-ischemic effects of *Ganoderma lucidum* polysaccharides (GLP) (Zhou et al., 2010), *Lycium barbarum* polysaccharide (LBP) (Wang T. et al., 2014; Zhao et al., 2017b), *Panax notoginseng* polysaccharides (PNP) (Dong et al., 2014), and *Cistanche deserticola* polysaccharides (CDP) (Liu et al., 2018) were associated with anti-oxidant activity and the regulation of Bcl-2 family members to maintain mitochondrial function and morphology. Furthermore, *Achyranthes bidentata* polypeptides (ABP) (Shen et al., 2010), astragalosides (Chiu et al., 2014), and phenolic acid extracts derived from *Sargentodoxa cuneata* (Bai M. et al., 2019) and *Salvia miltiorrhiza* (Hou et al., 2016; Yang et al., 2018; Wei Y. et al., 2016) also have potential anti-ischemic stroke effects. In conclusion, although the clinical treatment of ischemic stroke with a single herb is rare, a large number of definitive *in vitro* and *in vivo* clinical reports are sufficient to support further studies. However, the mechanism of some herbs with better efficacy proved by experiments is still in the preliminary stage, and the ischemic brain protection mechanism of anti-neuronal apoptosis is worthy of further exploration. More promisingly, some ethnic herbs for stroke prevention, such as Tibetan medicine saffron (Ochiai et al., 2007) and Mongolian medicine Eerdun Wurile (Gaowa et al., 2018), have also been gradually reported in recent years. In the early stage, our research group also revealed that the anti-hypoxia brain protection effect of the Tibetan medicine *Rhodiola crenulata* was related to the regulation of the HIF-1α/microRNA-210/ISCU12/COX10 signal pathway to improve mitochondrial energy metabolism, inhibit oxidative stress and mitochondrial apoptosis (Wang et al., 2019c). Although the medication law of ethnic medicine for prevention and
As research continues, massive active ingredients for treating stroke have been identified from herbal medicines. According to literature reports, we summarized 29 monomer compounds that may target to inhibit mitochondrial MPTP overopening-induced neuronal apoptosis, including alkaloids, flavonoids, terpenoids, and phenolic acids. **Figure 3** shows the structure information of these potential compounds. Tables 6 and 7 list the specific brain protective mechanisms of monomer compounds against ischemia-induced neuronal apoptosis. Notably, some of these compounds have been shown to regulate MPTP to improve ischemic stroke. The anti-oxidant and anti-inflammatory effects of hydroxy safflor yellow A (HSYA) and carboxyatractyloside could help to inhibit ischemia-induced MPTP opening and play a protective role against cerebral ischemia (Ramagiri and Taliyan, 2016). The anti-hypoxic effect of kaempferol was found to have a protective role against cerebral ischemia (Ramagiri and Taliyan, 2016).

### Monomers and Molecular Mechanisms in Regulating MPTP Openness of Ischemic Stroke

As research continues, massive active ingredients for treating stroke have been identified from herbal medicines. According to literature reports, we summarized 29 monomer compounds that may target to inhibit mitochondrial MPTP overopening-induced neuronal apoptosis, including alkaloids, flavonoids, terpenoids, and phenolic acids. **Figure 3** shows the structure information of these potential compounds. Tables 6 and 7 list the specific brain protective mechanisms of monomer compounds against ischemia-induced neuronal apoptosis. Notably, some of these compounds have been shown to regulate MPTP to improve ischemic stroke. The anti-oxidant and anti-inflammatory effects of hydroxy safflor yellow A (HSYA) and carboxyatractyloside could help to inhibit ischemia-induced MPTP opening and play a protective role against cerebral ischemia (Ramagiri and Taliyan, 2016). The anti-hypoxic effect of kaempferol was found to have a protective role against cerebral ischemia (Ramagiri and Taliyan, 2016).
related to inhibition of oxidative stress response, Ca\(^{2+}\) and ROS overproduction-evoked MPTP opening, and the transfer of mitochondrial Cyto-c to the cytoplasm, and thus increasing mitochondrial ATP supply and MMP (Sun et al., 2014). The authors further illuminated that GA could inhibit MPTP-induced apoptosis by regulating ERK-CypD axis, which may improve hypoxic brain edema (Panickar et al., 2015). Excitingly, recent study has further demonstrated that the anti-oxidative stress and apoptotic properties of trans-Angelicae Dahuricae Rhizoma (Zhu et al., 2014). Further studies have shown that the inhibitory effect of apoptosis was related to anti-inflammatory and down-regulation of MAPK signaling pathway (Zhao et al., 2017a). Similarly, alop勇ine (Ma et al., 2015), matrine, and oxymatrine (Zhao et al., 2015a; Zhao et al., 2015b; Liu Y. et al., 2019) may have the same protective effect on neuronal apoptosis under flow cytometry↓. Oxysophoridine could also suppress Ca\(^{2+}\) overload of neurons and maintain mitochondrial integrity (Li S. et al., 2018). Therefore, it is worth further focus on VDAC, one of the main components of MPTP, as an interesting target for stroke treatment.

In *in vivo* and *in vitro* studies have shown that oxisoyphoridine could regulate Bcl-2 family members, and thereby counteracting mitochondria-mediated apoptosis. Meanwhile, it was possible to suppress Ca\(^{2+}\) overload of neurons and maintain mitochondrial MMP by anti-oxidative stress and inhibiting the toxicity of neuronal excitatory amino acids (Chen et al., 2013; Wang et al., 2013; Zhao et al., 2013). Oxyphosphorine could also limit hyoxia-induced neuronal apoptosis by inhibiting Ca\(^{2+}\) and increasing MMP (Zhu et al., 2014). Further studies have shown that the inhibitory effect of apoptosis was related to anti-inflammatory and down-regulation of MAPK signaling pathway (Zhao et al., 2017a). Similarly, alop勇ine (Ma et al., 2015), matrine, and oxymatrine (Zhao et al., 2015a; Zhao et al., 2015b; Liu Y. et al., 2019) may have the same protective effect against ischemic neuron apoptosis. As a reversible selective inhibitor of true cholinesterase, huperzine A has been shown to inhibit mitochondrial complexes I–IV, a-ketoglutarate
dehydrogenase, and MMP decline after ischemia, which helps to eliminating excessive ROS and Ca^{2+} (Zheng et al., 2008). Considering the short in vivo half-life of tetramethylpyrazine, a novel compound containing tetramethylpyrazine and carnitine structures was synthesized. Further in vivo and in vitro results also confirmed that its anti-hypoxic brain protective effect was related to anti-oxidative stress and anti-inflammatory, ultimately maintaining the morphology and function of neurons and inhibiting neuronal apoptosis (Wang et al., 2017). Of course, there are other natural compounds that antagonize ischemia-infuriated morphological and functional disorders of brain mitochondria by regulating oxidative stress signals such as leonurine (Loh et al., 2010) and neferine (Wu C. et al., 2019).

Flavonoids resisting oxidative stress may drive the recovery of ischemia attacked neuron mitochondrial function, evidenced by increased mitochondrial biosynthesis and respiration, dampened Ca^{2+} production, and mitochondria edema, such as icariside II (Feng et al., 2018), as well quercetin and epicatechin in flavonols (Nichols et al., 2015). As an Nrf2 activator, mangiferin inhibited the nuclear translocation of two subunits of NF-κB, p65 and p50, and the superior antioxidant properties of mangiferin and morin inhibited Ca^{2+} overload and improved mitochondrial MMP, thus counteracting the lethal post-ischemic neuronal excitatory toxic damage and cascade apoptosis (Campos-Esparza et al., 2009). Other reports suggested that the protective effects of genistein (Qian et al., 2012), isorhamnetin (Li et al., 2016), and vitexin (Cui et al., 2019) against ischemia may involve both inflammation and inhibition of neuronal apoptosis. Most terpenoids also have antioxidant properties similar to those of alkaloids and flavonoids, which helped maintain mitochondrial morphology and respiratory function as well as ischemia-induced neuronal apoptosis, such as bilobalide (Schwarzkopf et al., 2013) and Swertiamarin (Wang et al., 2019b). Studies have shown that the treatment time window of asiatic acid can be maintained for at least 12 h, which is related to the improvement of MMP and the inhibition of mitochondrial Cyto-c release (Krishnamurthy et al., 2009). The balanced redox effect of ginsenoside Rd may contribute to the improvement of cerebral injury symptoms (Ye et al., 2011a). Further evidence showed that Rd could improve mitochondrial respiratory function and increase ATP production by reducing ROS production, thereby maintaining MMP and inhibiting neuronal apoptosis (Ye et al., 2011b), which was similar to dehydrocostuslactone’s protection of rat hippocampal slices from OGD/R-induced damage (Zhao et al., 2018a). Astragalosides IV may maintain mitochondrial function and inhibit OGD/R-induced cortical neuronal apoptosis by regulating PKA/CREB signaling pathway (Xue et al., 2019). In vivo and in vitro evidence suggested that Salvinorin A played an...
anti-apoptotic and anti-hypoxia protective role in brain involving the reduction of ROS and Ca\(^{2+}\) production in cerebrovascular endothelial cells, the activation of AMPK/Mfn2 signaling pathway, and ultimately maintenance of mitochondrial morphology and MMP (Dong et al., 2019). As an excellent natural biological cross-linking agent and a specific inhibitor of mitochondrial uncoupling protein 2 (UCP2), in vivo studies have shown that genipin could improve mitochondrial energy
metabolism by inhibiting UCP2-SIRT3 signaling pathway to mitigate oxidative stress injury and neuronal apoptosis after hypoxic brain injury (Zhao B. et al., 2019).

Other compounds such as taurine (Zhu et al., 2016) and echinacoside (Wei W. et al., 2019) could also regulate Bcl-2 family members through antioxidant stress, and inhibit mitochondrial apoptosis to improve hypoxic brain injury. Ischemic brain protection against neuronal apoptosis of phenolic acid compounds tetrahydroxystilbene glucoside (Wang et al., 2009), vanillin (Lan et al., 2019), curcumin (Zhang et al., 2017), and apocynin (Connell et al., 2012) may also further involved in the mechanism of anti-inflammatory, such as regulating the NF-kB and JNK, or targeting SIRT1. The antioxidant activity of quinones shikonin (Wang et al., 2010) and aloin (Chang R. et al., 2016), with a similar anti-cerebral ischemia action of rhein in our previous study (Zhao et al., 2018b), as well as phenylpropanoid compounds cinnamtannin D1 and trans-cinnamaldehyde (Panickar et al., 2015; Qi et al., 2016) from cinnamon might reduce the accumulation of Ca2+ and ROS, thus improving MMP to exert anti-ischemic neuron apoptosis. Through the above analysis of officially authorized drugs for the treatment of ischemic stroke, ethnic drug prescription, herbs, and monomer components, we found that most of them have the effect of anti-oxidative stress. The inhibition of overloaded Ca2+ and overproduced mtROS is the premise of drugs to reverse the decline of MMP after ischemia, improve mitochondrial respiratory function, and maintain the ATP supply of neurons. Although apoptosis might be the ultimate destination of neurons after ischemic stroke, we are pleasantly surprised to find that many adverse factors after ischemia might drive mitochondrial MPTP overopening. Meanwhile, we have previously discussed some potential proteins or oligomers that may be involved in regulating MPTP opening after cellular hypoxia, such as Bcl-2, Bax, Bcl-xl, and oligomer Bax/Bak of the Bcl-2 family. Through reviewing literatures, we also found that the above natural products could directly or indirectly inhibit MPTP overopening after ischemia. Furthermore, increased OMM permeability and collapsed mitochondrial membrane structures are inhibited. Ultimately, the integrity of the mitochondrial membrane and MMP are rescued, thus inhibiting the vicious cycle of excessive Ca2+ and mtROS production. As seen from the end results, caspase-dependent apoptosis triggered by the release of mitochondrial contents such as Cyto-c and AIF was blocked. Collectively, we have reason to believe that mitochondrial MPTP may be a potential target of natural products to inhibit neuronal apoptosis in treatment of ischemic stroke. Among the mechanisms, there may also be inflammation and oxidative stress signaling involved in MPTP opening and apoptosis. We summarized the mechanisms by which ethnic drugs may regulate MPTP to inhibit apoptosis of ischemic neurons, as shown in Figure 4. Among them, the mechanisms that have not been reported and elucidated still need to be further probed.

CONCLUSION AND FUTURE PROSPECTS

The literatures on targeted improvement of mitochondrial MPTP by ethnic medicine were reviewed systematically and purposefully. We were ecstatic to accept the trend that balanced mitochondrial MPTP was becoming a novel strategy for drug treatment of stroke (Briston et al., 2019). First, we identified that the process of stroke was associated with an abnormal over-opening of mitochondrial MPTP. Any factors that induced insufficient blood supply to the brain may lead to robust ROS, unbalanced intracellular Ca2+ homeostasis, decrease MMP, inflammation, and ERS. These detrimental events were doomed to be fatal to mitochondria and initiate changes in the three-dimensional conformation of mitochondrial MPTP, which would in turn aggravate the
| Agents | Objects | Gender | Weight (g) | Animal model | Dose | Time periods | Mechanisms | References |
|--------|---------|--------|------------|--------------|------|--------------|------------|------------|
| Asiatic acid | CS7BL/6 | male | 22–27 | PMCAO | 30, 75, and 163 mg/kg, i.g. | 1 h before and 3, 10, and 20 h after MCAO | Cyto-c, BBB permeability (IgG) | Krishnamurthy et al., 2009 |
| Ginsenoside | SD | male | 270–320 | MCAO | 50 mg/kg, i.p. | 20 min before MCAO | MMP, aconitase, mitochondrial complexes I–IV; ROS, lactate/pyruvate ratio, cleaved Caspase-3, Cyto-c, AIF, SOD1, GSH-Px, mitochondrial Cyto-c, MDA1, mtROS, cytosolic Cyto-c, Caspase-3, TUNEL-positive neurons, p-NF-κB p65 subunit, p-IκBα, SOD1, GSH-Px, Bcl-2, MDA1, Caspase-3, Bax, TUNEL-positive neurons | Ye et al., 2011b |
| Genistein | CS7/BL6J | male | 24–28 | MCAO (1 h)/R (24 h) | 2.5, 5, and 10 mg/kg, i.g. | Pretreatment once daily for 2 w | Wang et al., 2013 |
| Oxyphosphoridine | ICR | male | 20–25 | MCAO (2 h)/R (24 h) | 62.5, 125, and 250 mg/kg, i.p. | Pretreatment once daily for 1 w | Wu et al., 2019 |
| Echinacoside | SD | both | 12–17 | MCAO | 40, 80, and 160 mg/kg, i.p. | Every 12 h after operation, a total of 4 times | Sun et al., 2014 |
| Gallic acid | SD | male | 250–300 | MCAO (2 h)/R (24 h) | 25 and 50 mg/kg, i.v. | 20 min before MCAO | MMP, mitochondrial Cyto-c; MDA1, ROS1, cytosolic Cyto-c, TUNEL-positive neurons | Sun et al., 2017 |
| Oxymatrine | SD | both | — — | MCAO | 30, 60, and 120 mg/kg, i.p. | Every 12 h after operation, a total of 2 times | Zhao et al., 2015a |
| Matrine | ICR | male | 20–25 | MCAO (2 h)/R (24 h) | 7.5, 15, and 30 mg/kg, i.v. | Pretreatment once daily for 1 w | Zhao et al., 2015b |
| Taurine | SD | both | — — | MCAO | 30, 60, and 120 mg/kg, i.p. | Every 12 h after operation, a total of 2 times | Zhu et al., 2016 |
| HSYA | Wistar | male | 220–250 | MCAO (1 h)/R (24 h) | 8 mg/kg, i.v. | After reperfusion | GSH1, CAT1, MDA1, TNF-α1, MPTP opening, Bcl-2, Sirt1, MPP+; p53, Bax, IL-6, Bax, TNF-α1, ROS1, VCOC1, cytoplasmic and nuclear EndoG4, ROS1, MPTP opening, TUNEL-positive neurons | Ramagiri and Tallyan, 2016 |
| Curcumin | Wistar | male | 180–200 | MCAO/R | 25 mg/kg, i.p. | 15 min before MCAO/R | Zhao et al., 2018 |
| Picroside II | Wistar | male | 240–260 | MCAO (2 h)/R (24 h) | 20 mg/kg, i.p. | 3 days following MCAO/R | Zhao et al., 2018b |
| Rhein | SD | male | 260–300 | MCAO (2 h)/R (72 h) | 25, 50, and 100 mg/kg, i.g. | 3 days following MCAO/R | ATP1, SOD1, GSH1, UCP21, SIRT31, NAD+/NADH, LDH1, cleaved Caspase-3, TUNEL-positive neurons | Zhao et al., 2019 |
| Genipin | CS7BL/6 | male | 25–30 | MCAO (1 h)/R (24 h) | 50 mg/kg, i.g. | Pretreatment once daily for 3 d | Wang et al., 2019b |
| Swertiamarin | ICR | male | 20–25 | MCAO (2 h)/R (24 h) | 25, 100, and 400 mg/kg, i.p. | Pretreatment once daily for 1 w | Wang et al., 2019b |

†, upgrade; ‡, downgrade.
production of mitochondrial ROS, mitochondrial edema, the booming cytoplasmic Ca^{2+}, the decline of MMP, and the reduction of ATP synthesis. To sum up, all these adverse biological events that caused the loss of the function of mitochondrial bilayer barrier would inevitably disrupt the material transfer between mitochondrial matrix and cytoplasm. Consequently, the activated mitochondrial dependent apoptosis was triggered according to an inherent set of biological

| Agents                              | Cell lines | Model               | Dose                  | Time periods              | Mechanisms                                                                 | References |
|-------------------------------------|------------|---------------------|-----------------------|---------------------------|----------------------------------------------------------------------------|------------|
| Asiatic acid                        | HT-22      | OGD (5 h/R (24 h))  | 1 and 10 μg/ml        | Posttreatment for 24 h    | MMP↑, Cyto-c↓                                                              | Krishnamurthy et al., 2009 |
| Tetrahydroxystilbene glucoside      | PCN of neonatal SD rats | OGD (5% CO2, and 95% N2, 2 h/R (24 h)) | 25 μM                    | Pretreatment for 24 h    | MMP↑, SIRT1↑, Bcl-2/Bax↓, LDH↓, ROS↓, p-JNK↑, INOS↑, nuclear p65↓, Ca^{2+}↑, Hoechst 33342 positive staining↓ | Wang et al., 2009 |
| Mangiferin and Morin                | PCN of embryonic SD rats | OGD (5% CO2, 94% N2, and 1% O2, 6 h/R (24 h)) | 1–10 μM                  | During and after glutamate exposure | SOD↑, CAT↑, p-Akt↑, cytoplasmic p65↑, MMP↑, Calpain↑, p-Erk1/2↑, nuclear p65↑, AIF↓, Bax↑, ROS↓ | Campos-Esparza et al., 2009 |
| Trans resveratrol                   | PC12       | OGD (5% CO2, 94% N2, and 1% O2, 6 h/R (24 h)) | 5, 10, and 25 μM        | 24 h before/ post OGD    | Bcl-2↑, GSH↑, Bax↑, HIF-1α↑, Caspase-3↑, ROS↓, LPO↑ | Agrawal et al., 2011 |
| Oxyssorphorine                      | PHN of neonatal SD rats | OGD (2 h/R (24 h)) | 5, 20, and 80 μM       | OGD (2 h/R (24 h))       | Bcl-2/Bax↑, Caspase-3/8/9↑, Cyto-c↑, Hoechst-33342 fluorescence intensity↑ | Chen et al., 2013; Zhao et al., 2013 |
| Gallic acid                         | SH-SYSY    | Hypoxia (Na2SO4, 2 h/R (2 h)) | 0.1, 1, and 10 μM      | Pretreatment for 24 h    | MMP↑, ATP↑, oxygen consumption↑, MDA↑, intracellular ROS↓, miROS↓, MPTP opening↓ | Sun et al., 2014 |
| Oxyssophorcarpine                   | PHN of neonatal SD rats | OGD (5% CO2, and 95% N2, 2 h/R (24 h)) | 1, 2, and 5 μmol/L      | During reperfusion period | MMP↑; LDH↓, Ca^{2+}↓, Caspase-3/12↓ | Zhu et al., 2014 |
| Epicetechin and Quercetin           | PHN of embryonic C1D1 mice | OGD (5% CO2, and 5% H2O, and 90% N2, 5 min/R (1.5 h)) | 0.1-10 μM               | OGD (2 h/R (24 h))       | MPTP opening | Nichols et al., 2015 |
| Aloe vera                           | PHN of neonatal SD rats | OGD (5% CO2, and 95% N2, 2 h/R (24 h)) | 25, 50, and 100 μmol/L | During reperfusion period | CAT↑, SOD↑, GSH-px↑, T-AOC↓, MMP↑, LDH↓, Ca^{2+}↓, MDA↑, ROS↑, Hoechst 33342 positive staining↓ | Ma et al., 2015 |
| Alpin                                 | PC12       | OGD (5% CO2, and 95% N2, 4 h/R (24 h)) | 40, 20, and 10 μg/ml   | During OGD/R period     | MMP↑, Bcl-2↑, SOD↑, LDH↓, MDA↑, ROS↓, Ca^{2+}↓, Bax↑, Caspase-3↑, Hoechst 33342 positive staining↑, apoptosis under flow cytometry↑ | Chang R., et al., 2016 |
| Kaempferol                           | PCN of 17-d embryonic rats | OGD (2 h) | 10 μM      | Before OGD                       | OCFT↑, p-Akt↑, MMP↑, p-Drp1/Dmp1↑, AIF↑, ATPI↑, HK-lt↑, LC3 II↑ ratios↑, mitochondrial Cyto-cytosolic Cyto-c↑, ROS↑, Ca^{2+}↑, SDH↑↑, apoptosis under flow cytometry↑, MPTP opening↑ | Wu B., et al., 2017 |
| Dehydrocostuslactone                | hippocampal slices of SD rats | OGD (5% CO2, and 95% N2, 0.5 μmol/L h/R (1 h)) | 1, 5, and 10 μM         | During OGD/R period     | LC3 II↑ ratios↑, Bcl-2↑, LDH↑, Bax↑, Cyto-c↓, Apat-1↑, Caspase-3/7/9↑, p62↑ | Zhao et al., 2018a |
| Icariside II                        | PC12       | OGD (5% CO2, and 95% N2, 2 h/R (24 h)) | 12.5, 25, and 50 μM   | Posttreatment for 24 h    | nuclear Nrf2↑, NQO-1↑, HO-1↑, Bcl-2/Bax↑, SIRT3↑, LDH↑, and MMP↑, LDH↑, ROS↑, cytoplasmic Nrf2↑, Keap1↓, cleaved Caspase-3↑, TUNEL-positive neurons↑ | Feng et al., 2018 |
| Astragaloside IV                    | PCN of 18-d embryonic rats | OGD (1% CO2, 5% CO2, 3 h/R (24 h)) | 6.25, 12.5, and 25 μmol/L | During OGD/R period     | p-PKA/pKA and p-CREB/CREB↑, ATPI↑, LDH↑, Bax↑, cleaved Caspase-3↑, ROS↓ | Xue et al., 2019 |
| Oxyymatrine                         | PHN of newborn SD rats | OGD (5% CO2, and 95% N2, 2 h/R (24 h)) | 0.2, 1, and 5 μg/ml   | During reperfusion period | MCL-1↑, Bcl-2↑, p-Akt↑, p-PI3K↑, p-GSK3β↑, MMP↑, LDH↑, Ca^{2+}↑, Caspase-3↑, NR2B↓ (NMDAR1)↑, TUNEL-positive neurons↑, neuronal apoptosis under flow cytometry↑ | Liu Y. et al., 2019 |
| Salvinorin A                         | HBMECs     | OGD (5% CO2, and 95% N2, 6 h/R (24 h)) | 5 μmol/L            | During reperfusion period | p-AMPK↑, Min2↑, ATP↑, MMP↑, ROS↓, Ca^{2+}↑, apoptosis under flow cytometry↑ | Dong et al., 2019 |

†, upgrade; ↓, downgrade.

**TABLE 7**: The in vitro mechanism underlying the inhibition of MPTP opening-induced neuronal apoptosis by monomeric compounds in the treatment of ischemic stroke.
procedures. And this process was regularly and strictly executed by mitochondria-emitted apoptosis signal, and delivered step by step. For instance, ischemia-induced MPTP opening leaded to the translocation of Cyto-c from mitochondrial matrix into cytoplasm, and binding with Apaf-1 and Caspase-9 to form apoptosome, thereby activating caspase-dependent programmed cell death pathways in ischemic/anoxic neurons. Secondly, we found piece by piece that ethnic drug prescriptions, herbal medicine, and monomer components could participate in regulation of excessive MPTP opening induced-ischemic neuronal apoptosis from different perspectives. We therefore concluded that mitochondrial MPTP, a very considerably intermediate link in apoptosis signaling, might be a novel target for natural products in treatment of stroke.

However, by weighing the pros and cons, the following aspects should be worthy to further optimization considering the anti-apoptotic brain protection effect of ethnic drugs through regulation of mitochondrial MPTP. First, the complexity and uncertainty of active ingredients penetrating blood brain barrier (BBB). Current methods for identifying active ingredients included high performance liquid chromatography (HPLC), mass spectrum, gas chromatography-mass spectrometer (GC-MS), or liquid chromatograph-mass spectrometer (LC-MS). However, the key problem lay in the selection and preprocessing of samples for content determination: the original herbs or prescription extracted by simple decoction, ultrasound or different proportions of organic reagents, animal serum or brain tissue homogenate after administration. Any test based on those ideas would simply identify specific monomer compounds contained in certain prescriptions or extracts. However, the premise of drug efficacy was to achieve a certain concentration in target organs or tissues such as specific brain regions to stimulate the transmission of anti-apoptotic protective signals. Slightly regretfully, the qualitative or quantitative identification methods mentioned above cannot completely represent the concentration of drug enrichment in cerebral ischemic regions. For this existing and confronting problems, we proposed that a microdialysis device coupled HPLC/MS would be a potential platform for screening active ingredients (Reyes-Garcés et al., 2019) or changeable pH value of brain microenvironment (Su and Ho, 2019). Moreover, distribution concentrations of different small molecule drugs targeting distinct brain regions could be dynamically presented in real time and in vivo by an integrated platform of high resolution laser confocal microimaging coupled with brain MS imaging (He et al., 2019; Liu C. et al., 2019). Finally, a multi-dimensional image of drug distribution in brain tissue was visually and stereoscopically constructed. Second, the rationality of in vivo and in vitro simulation of clinical stroke model in light of the complexity of BBB tissue structure (Sweeney et al., 2019). Currently, diverse in vivo stroke models for cerebral ischemia, or in vitro OGD/R-

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**FIGURE 4 |** A panoramic view of natural products inhibiting MPTP opening-induced neuronal apoptosis in the treatment of ischemic stroke. Any adverse stimuli after ischemic stroke could favor MPTP opening. However, natural products that inhibit MPTP opening could further prevent neuronal inflammation after ischemia, oxidative stress injury, and mitophagy, and finally repress ischemic neuron apoptosis.
induced hippocampal slices or different neuron injury models, which were widely accepted and acquiescent, cannot reproduce the scene of changes in brain tissue structure and the specific molecular-mediated damage mechanisms yet. Therefore, the above existing stroke models needed to be further discussed. However, it was encouraging to note that our research group had successfully established *in vitro* co-culture models of cerebrovascular endothelial cell, astrocytes, and pericytes to simulate BBB (the data have not yet been published), referring to the organ-like models of multiple neurons co-culture or BBB previously reported (Bergmann et al., 2018). Of course, through establishment of *in vitro* neurovascular unit (NVU), we also strived to achieve real-time and rapid evaluation of natural small molecule compounds passing BBB, and to screen the quality markers of ethnic drugs and functional protein targets on a coupled microfluidic chip-mass spectrometry (MC-MS) platform (Wang et al., 2019d).

Third, the mechanisms of small molecular compounds acting on mitochondrial MPTP to inhibit apoptosis after ischemic stroke were unsophisticated. According to what we have learned, the conventional means demonstrating the interrelationship between drugs and MPTP were limited to the following. After intervention with MPTP inhibitors or agonists, conventional western blot, immunohistochemistry/fluorescence (Bonora et al., 2016), flow cytometry, and qRT-PCR were employed to evaluate the effect of drugs on changes in protein and gene expression that made up MPTP, such as VDAC and ANT. In addition, gene editing such as plasmids or viruses transfection of target gene vectors to overexpress or silence the target gene, or to completely knock out or down the target gene and observe the effect of drugs on MPTP were also some popular molecular biology methods. Certain proteins or protein complexes such as Bax/Bak dimerization, mtROS, oxidative stress, and inflammatory factors could regulate MPTP opening-induced cell apoptosis, thus providing indirect evidence for drug regulation of MPTP. The more intuitive evidence might be to detect some of triggering hallmarks after MPTP opening, such as mitochondrial swelling, decreased MMP and ATP production, and detection of fluorescent labeled cytoplasmic Ca2+ surge. However, none of the above methods could provide direct evidence of drug-MPTP-apoptosis. That is, it cannot be visualized that drugs confined MPTP opening, and thus inhibiting cell apoptosis. The deficiencies of the above mechanisms investigation included the limited understanding of MPTP and the limitations of current molecular imaging technologies. Therefore, more efforts were needed to explore the molecular basis and regulatory mechanism of MPTP. We also had reason to believe that the laser confocal high intentionality live cell real-time imaging and analysis system would

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**FIGURE 5** | Conceptual flowchart of combined multiple techniques for MPTP regulation by natural products on apoptosis of ischemic neurons. Mitochondrial MPTP is a novel target for the treatment of ischemic stroke. Determination of the distribution of natural products in distinct brain regions, reasonable in vivo and in vitro stroke models, and advanced MPTP imaging technologies will be conducive to the development of ethnic drugs targeting MPTP.
be a robust alternative for probing drug targeted regulation of MPTP. Moreover, patch-clamp combined with two-photon living cell imaging technology also had potential prospects for detection of prophylaxis and treatment of ethnic drugs on post-stroke mitochondrial MMP and Ca\(^{2+}\) or other ion levels (Kislin et al., 2017; Wilson et al., 2018; Zhang et al., 2019c). In conclusion, we were optimistic that abnormal opening of mitochondrial MPTP-induced apoptosis would become a potential target for stroke treatment by ethnic medicine. Further, we conceived and constructed the systematic process and program of drugs regulating mitochondrial MPTP to inhibit apoptosis in ischemic stroke, as shown in Figure 5. However, objectively speaking, no matter how many preclinical investigations were merely paving the way for screening mitochondrial MPTP targeted candidates, clinical trials with large samples and multi-center joint evaluation of the clinical efficacy of candidates were still necessary to be carried out.

**AUTHOR CONTRIBUTIONS**

XW conceived the study. YL, JS, RW, JB, YH, YZe, XM, and YZh reviewed and summarized the literatures. XW wrote the manuscript and drew all the figures. XW, ZW, and XM supervised the study and gave final approval of the version to be published. The final version of the manuscript was read and approved by all authors.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00352/full#supplementary-material

**REFERENCES**

Agarwal, A., Wu, P. H., Hughes, E. G., Fukaya, M., Tischfeld, M. A., Langseth, A. J., et al. (2017). Transient Opening of the Mitochondrial Permeability Transition Pore Induces Microdomain Calcium Transients in Astrocyte Processes. *Neuron* 93, 587–605.e7. doi: 10.1016/j.neuron.2016.12.034

Agrawal, M., Kumar, V., Kashyap, M. P., Khanna, V. K., Randhawa, G. S., and Pant, A. B. (2011). Ischemic insult induced apoptotic changes in PC12 cells: protection by trans resveratrol. *Eur. J. Pharmacol.* 666, 5–11. doi: 10.1016/j.ejphar.2011.05.015

Andrabi, S. S., Parvez, S., and Tabassum, H. (2017). Progesterone induces neuroprotection following reperfusion-promoted mitochondrial dysfunction after focal cerebral ischemia in rats. *Dis. Model. Mech.* 10, 787–796. doi: 10.1242/dmm.025692

Andrabi, S. S., Ali, M., Tabassum, H., Parvez, S., and Parvez, S. (2019). Pimipexole prevents ischemic cell death via mitochondrial pathways in ischemic stroke. *Dis. Model. Mech.* 12, 1–11. doi: 10.1242/dmm.033860

Arnould, D., Gaume, B., Karbowskii, M., Sharpe, J. C., Cecconi, F., and Youle, R. J. (2003). Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. *EMBO J.* 22, 4385–4399. doi: 10.1093/emboj/cdg423

Bai, J., Gao, Y., and Gao, Y. H. (2019). Effect of Pei Yuan Tong Nao capsules on neuronal function and metabolism in cerebral ischemic rats. *J. Ethnopharmacol.* 238, 111837. doi: 10.1016/j.jep.2019.111837

Bai, M., Liu, B., Peng, M., Jia, J., Fang, X., and Miao, M. (2019). Effect of Sargentodoxa cuneata total phenolic acids on focal cerebral ischemia reperfusion injury rats model. *Saudi J. Biol. Sci.* 26, 569–576. doi: 10.1016/j.sjbs.2018.11.019

Baines, C. P., and Gutiérrez-Aguilar, M. (2018). The still uncertain identity of the channel-forming unit(s) of the mitochondrial permeability transition pore. *Cell Calcium* 73, 121–130. doi: 10.1016/j.ceca.2018.05.003

Bergmann, S., Lawler, S. E., Qu, Y., Fadzen, C. M., Wolfe, J. M., Regan, M. S., et al. (2018). Blood-brain-barrier organoids for investigating the permeability of CNS therapeutics. *Nat. Protoc.* 13, 2827–2843. doi: 10.1038/s41596-018-0066-x

Bernardi, P., Rasola, A., Forte, M., and Lippe, G. (2015). The Mitochondrial Permeability Transition Pore: Channel Formation by F-ATP Synthase, Integration in Signal Transduction, and Role in Pathophysiology. *Physiol. Rev.* 95, 1111–1155. doi: 10.1152/physrev.00001.2015

Bonora, M., and Pinton, P. (2019). A New Current for the Mitochondrial Permeability Transition. *Trends Biochem. Sci.* 44, 559–561. doi: 10.1016/j.tibs.2019.04.009

Bonora, M., Morganti, C., Morciano, G., Giorgi, C., Wieckowski, M. R., and Pinton, P. (2016). Comprehensive analysis of mitochondrial permeability transition pore activity in living cells using fluorescence-imaging-based techniques. *Nat. Protoc.* 11, 1067–1080. doi: 10.1038/nprot.2016.064

Bora, K. S., and Sharma, A. (2010). Neuroprotective effect of Artemisia absinthium L. @ on focal ischemia and reperfusion-induced cerebral injury. *J. Ethnopharmacol.* 129, 403–409. doi: 10.1016/j.jep.2010.04.030

Brenner, C., and Moulin, M. (2012). Physiological roles of the permeability transition pore. *Circ. Res.* 111, 1237–1247. doi: 10.1161/CIRCRESAHA.112.265942

Briston, T., Selwood, D. L., Szabadkai, G., and Duchen, M. R. (2019). Mitochondrial Permeability Transition: A Molecular Lesion with Multiple Drug Targets. *Trends Pharmacol. Sci.* 40, 50–70. doi: 10.1016/j.tips.2018.11.004

Cai, Y. M., Zhang, Y., Zhang, P. B., Zhen, L. M., Sun, X. J., Wang, Z. L., et al. (2016). Neuroprotective effect of Shengqi Fuzheng injection pretreatment in aged rats with cerebral ischemia/reperfusion injury. *Neural Regen. Res.* 11, 94–100. doi: 10.11031637-5374.175052

Cai, M., Yang, Q., Li, G., Sun, S., Chen, Y., Tian, L., et al. (2017). Activation of cannabinoid receptor 1 is involved in protection against mitochondrial dysfunction and cerebral ischaemic tolerance induced by isoflurane preconditioning. *Br. J. Anaesth.* 119, 1213–1223. doi: 10.1093/bja/aex267

Campbell, B. C. V., De Silva, D. A., Macleod, M. R., Coutts, S. B., Schwamm, L. H., Davis, S. M., et al. (2019). Ischaemic stroke. *Nat. Rev. Dis. Primers* 5, 70. doi: 10.1038/s41572-019-0114-8

Campos-Esparza, M. R., Sánchez-Gómez, M. V., and Matute, C. (2009). Molecular mechanisms of neuroprotection by two natural antioxidant polyphenols. *Cell Calcium* 45, 358–368. doi: 10.1016/j.cca.2008.12.007

Cao, G., Zhou, H., Jiang, N., Han, Y., Hu, Y., Zhang, Y., et al. (2016). YiQiFuMai Powder Injection Ameliorates Cerebral Ischemia by Inhibiting Endoplasmic Reticulum Stress-Mediated Neuronal Apoptosis. *Oxid. Med. Cell Longev.* 2016, 1–14. doi: 10.1155/2016/5493279
Chang, J., Yao, X., Zou, H., Wang, L., Lu, Y., Zhang, Q., et al. (2016). BDNF/PI3K/ Akt and Nogo-A/RhoA/ROCK signaling pathways contribute to neurorestorative effect of Hoshuaisen against cerebral ischemia injury in rats. J. Ethnopharmacol. 194, 1032–1042. doi: 10.1016/j.jep.2016.11.005

Chang, R., Zhou, R., Qi, X., Wang, J., Wu, F., Yang, W., et al. (2016). Protective effects of aloe on oxygen and glucose deprivation-induced injury in PC12 cells. Brain Res. Bull. 121, 75–83. doi: 10.1016/j.brainresbull.2016.01.001

Chen, R., Li, Y. X., Jiang, N., Ma, N. T., Zhe, Q. L., Hao, Y. J., et al. (2013). Anti-apoptotic and neuroprotective effects of oxysophoridine on cerebral ischemia both in vivo and in vitro. Plant. Med. 79, 916–923. doi: 10.1055/s-0032-1328705

Chen, L., Zhao, Y., Zhang, T., Dang, X., Xie, R., Li, Z., et al. (2014). Protective effect of Sheng Nao Kang decoction on focal cerebral ischemia-reperfusion injury in rats. J. Ethnopharmacol. 151, 228–236. doi: 10.1016/j.jep.2013.10.015

Chen, Z. Z., Gong, X., Guo, Q., Zhao, H., and Wang, L. (2019). Bu Yang Huan Wu of Sheng-Nao-Kang decoction on focal cerebral ischemia-reperfusion injury in a rat model of ischemia/reperfusion. Brain Res. Bull. 132, 83–92. doi: 10.1016/j.brainresbull.2016.01.001

Chou, S. M., Zhao, M. H., Shen, P. P., Liu, X. P., Sun, Y., and Feng, J. C. (2016). Neuroprotective Effect of Salvianolic Acids against Cerebral Ischemia/Reperfusion Injury. Int. J. Mol. Sci. 17, 1190. doi: 10.3390/ijms17071190

Chou, Y., Qieni, X., Li, N., Bai, J., Li, R., Gongbao, D., et al. (2020). Longzhibu disease and its therapeutic effects by traditional Tibetan medicine: Ershi-wei Chexiang pills. J. Ethnopharmacol. 249, 114246. doi: 10.1016/j.jep.2019.114246

Cui, Y. H., Zhang, X. Q., Wang, N. D., Zheng, M. D., and Yan, J. (2019). Vitexin of Dihuang Yinzi (DY) plays neuroprotective and anti-dementia role in rats of ischemic brain injury. J. Ethnopharmacol. 121, 444–450. doi: 10.1016/j.jep.2008.09.035

Huang, Q., Lan, T., Lu, J., Zhang, H., Zhang, D., Lou, T., et al. (2018). DiDang Tang Inhibits Endoplasmic Reticulum Stress-Mediated Apoptosis Induced by Oxygen Glucose Deprivation and Intracerebral Hemorrhage Through Blockade of the GRP78/IRE1/PERK Pathways. Front. Pharmacol. 9, 14–23. doi: 10.3389/fphar.2018.01423

Kislin, M., Sword, J., Fomitcheva, I. V., Croom, D., Pryazhnikov, E., Lihavainen, E., et al. (2017). Reversible Disruption of Neuronal Mitochondria by Ischemic and Reperfusion Injury via Connexin 43/Calpain II/Bax/Caspase-3 pathway in rat. J. Neurochem. 139, 162–170. doi: 10.1111/jnch.13903

Dong, J., Deng, Y., Gao, J., Liu, X., Chu, J., and Shu, Y. (2014). Neuroprotective effect of Panax notoginseng polysaccharides against focal cerebral ischemia/reperfusion injury in rats. Int. J. Biol. Macromol. 63, 177–180. doi: 10.1016/j.jbiomac.2013.10.034

Gao, S., Bao, N., Da, M., Qiburi, Q., Ganbold, T., Chen, L., et al. (2018). Traditional Mongolian medicine Eerdun Wurile improves stroke recovery through regulation of gene expression in rat brain. J. Ethnopharmacol. 222, 249–260. doi: 10.1016/j.jep.2018.05.011

Hånell, A., Greer, J. E., McGinn, M. J., and Povlishock, J. T. (2015). Traumatic brain injury-induced axonal phenotypes react differently to treatment. Acta Neuropathol. 129, 317–332. doi: 10.1007/s00401-014-1376-x

Hui, X., Yang, S., Yangqiong, Z., Qiang, J., Defeng, L., Yi, Z., et al. (2016). Identification of key active constituents of Buchang Naoxionting capsules with therapeutic effects against ischemic stroke by using an integrative pharmacology-based approach. Mol. Biosyst. 12, 233–245. doi: 10.1039/c5mb00460h

He, H., Qin, L., Zhang, Y., Han, M., Li, J., Liu, Y., et al. (2019). 3,4-Dimethoxyamphetamine as a Novel Matrix for Enhanced In Situ Detection and Imaging of Low-Molecular-Weight Compounds in Biological Tissues by MALDI-MSI. Anal. Chem. 91, 2634–2643. doi: 10.1021/acs.analchem.8b03322

Hou, S., Zhao, M. H., Shen, P. P., Liu, X. P., Sun, Y., and Feng, J. C. (2016). Neuroprotective Effect of Salvianolic Acids against Cerebral Ischemia/Reperfusion Injury. Int. J. Mol. Sci. 17, 1190. doi: 10.3390/ijms17071190

Khalifa, M. I., Khalil, H., Sargenti, A., Marziano, L., Terada, N., et al. (2019). Inhibition of mitochondrial permeability transition by deletion of the ANT family and CypD. Sci. Adv. 5, eaw4597. doi: 10.1126/sciadv.aaw4597

Khouyr, N., X., Stegelm, S. D., Jackson, C. W., Koronowski, K. B., Dave, K. R., et al. (2019). Resveratrol Preconditioning Induces Genomic and Metabolic Adaptations within the Long-Term Window of Cerebral Ischemic Tolerance Leading to Bioenergetic Efficiency. Mol. Neurobiol. 56, 4595–4565. doi: 10.1007/s12035-018-1380-6

Ku, S. H., Chung, D. K., Lee, Y. J., Song, C. H., and Ku, S. K. (2016). Neuroprotective effects of Dansgui-Jiyak-San on rat stroke model through antioxidant/antiapoptotic pathway. J. Ethnopharmacol. 188, 123–133. doi: 10.1016/j.jep.2016.06.040

Kislis, M., Sword, J., Fomitcheva, I. V., Croom, D., Pryazhnikov, E., Li, V., et al. (2017). Reversible Disruption of Neuronal Mitochondria by Ischemic and Traumatic Injury Revealed by Quantitative Two-Photon Imaging in the Neocortex of Anesthetized Mice. J. Neurosci. 37, 333–348. doi: 10.1523/JNEURSCI.1510-16.2016

Krishnamurthy, R. G., Senut, M. C., Zenlke, D., Min, J., Frenkel, M. B., Greenberg, E. J., et al. (2009). Asatic acid, a pentacyclic triterpene from Gentella asiatica, is neuroprotective in a mouse model of focal cerebral ischaemia. J. Neurosci. Res. 87, 2541–2550. doi: 10.1002/jnr.22071

Kumari, S., Mehta, S. L., Milleide, G. Z., Huang, X., Li, H., and Li, P. A. (2016). Ubisol-Q10 Prevents Glutamate-Induced Cell Death by Blocking Mitochondrial Fragmentation and Permeability Transition Pore Opening. Int. J. Biol. Sci. 12, 688–700. doi: 10.7150/ijb.13589

Hou, R., Zhang, Y., Xiang, J., Zhang, W., Wang, H. G., Li, W. W., et al. (2014). Xiao-Xu-Ming decoction preserves mitochondrial integrity and reduces apoptosis after focal cerebral ischemia and reperfusion via the mitochondrial p53 pathway. J. Ethnopharmacol. 151, 307–316. doi: 10.1016/j.jep.2013.10.042

Lan, X., B., Wang, Q., Yang, J. M., Ma, L., Zhang, W. J., Zheng, P., et al. (2019). Neuroprotective effect of Vanillin on hypoxic-ischemic brain damage in neonatal rats. Biomed. Pharmacother. 118, 109–196. doi: 10.1016/j.biopharm.2019.109196
Lee, J. J., Hsu, W. H., Yen, T. L., Chang, N. C., Luo, Y. J., Hsiao, G., et al. (2011). Traditional Chinese medicine, Xue-Fu-Zhu-Yu decoction, potentiates tissue plasminogen activator against thrombembolic stroke in rats. J. Ethanopharmacol. 134, 824–830. doi: 10.1016/j.jep.2011.01.033

Li, H., Deng, C. Q., Chen, B. Y., Zhang, S. P., Liang, Y., and Luo, X. G. (2009). Total saponins of Panax notoginseng modulate the expression of caspases and attenuate apoptosis in rats following focal cerebral ischemia-reperfusion. J. Ethanopharmacol. 121, 412–418. doi: 10.1016/j.jpetho.2008.10.042

Li, L. H., Wang, J. S., and Kong, L. Y. (2013). Protective effects of shengmai san and its three fractions on cerebral ischemia-reperfusion injury. Chin. J. Nat. Med. 11, 222–230. doi: 10.3887/SJ.13560020-5

Li, W., Li, P., Liu, Z., Wu, Q., He, P., Chen, Z., and Dai, H. (2016). The protective role of isorhamnetin on human brain microvascular endothelial cells from cytotoxicity induced by methylglyoxal and oxygen-glucose deprivation. J. Ethnopharmacol. 173, 196–203. doi: 10.1016/j.jep.2016.01.016

Li, M., Zhai, X., Wang, T., Ge, K., Zhao, J., Cong, W., et al. (2018). Picroside II attenuates apoptosis in rats following focal cerebral ischemia-reperfusion. J. Ethnopharmacol. 211, 222–30. doi: 10.1016/j.jep.2016.01.016

Lee, J. J., Hsu, W. H., Yen, T. L., Chang, N. C., Luo, Y. J., Hsiao, G., et al. (2011). Mechanistic Role of mPTP in Ischemia-Reperfusion Injury. Circ. Res. 109, 151–161. doi: 10.1161/CIRCRESAHA.110.296891

Li et al. Ethnomedicine and MPTP in Apoptosis

Matsuno, S., Friberg, H., Ferrand-Drake, M., and Wieloch, T. (1999). Blockade of the mitochondrial permeability transition pore diminishes infarct size in the rat after transient middle cerebral artery occlusion. J. Cereb. Blood Flow Metab. 19, 736–741. doi: 10.1097/00004467-199907000-00002

Mattiaison, G., Shalamoo, M., Gido, G., Mathi, K., Tomasevic, G., Yi, S., et al. (2003). Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. Nat. Med. 9, 1062–1068. doi: 10.1038/nm903

McArthur, K., Whitehead, L. W., Hedleston, J. M., Li, L., Padman, B. S., Oorschot, V., et al. (2018). BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. Science 359, 883, eaao6047. doi: 10.1126/science.aao6047

Mehta, S. L., and Li, P. A. (2009). Neuroprotective role of mitochondrial uncoupling protein 2 in cerebral stroke. J. Cereb. Blood Flow Metab. 29, 1069–1078. doi: 10.1038/jcbfm.2009.4

Meng, X., Xie, W., Xu, Q., Liang, T., Xu, X., Sun, G., et al. (2018). Neuroprotective Effects of Radix Scrophulariae on Cerebral Ischemia and Reperfusion Injury via MAPK Pathways. Molecules 23, 2401. doi: 10.3390/molecules2302401

Nichols, M., Zhang, J., Polster, B. M., Eustondeo, P. A., Thirumaran, A., Pavlov, E. V., et al. (2015). Synergistic neuroprotection by epicatechin and quercetin: Activation of convergent mitochondrial signaling pathways. Neuroscience 308, 75–94. doi: 10.1016/j.neuroscience.2015.09.012

Ochiai, T., Shimeno, H., Mishima, K., Iwaski, K., Fujitani, M., Matsumoto, S., and Prentice, H., Modi, J. P., and Wu, J. Y. (2015). Mechanisms of Neuronal Protection and Regulation of Mitochondrial Complex I Activity by the Bcl-xL/Bcl-2-Related Factor 2. Cell Death Differ. 12, 713–723. doi: 10.1038/sj.cdd.4401638

Pan, R., Cai, J., Zhan, L., Guo, Y., Huang, R. Y., Li, X., et al. (2017). Buyang Huidi decoction facilitates neurorehabilitation through an improvement of synaptic plasticity in cerebral ischemic rats. BMC Complement. Altern. Med. 17, 173. doi: 10.1186/s12906-017-1680-9

Panel, M., Ruiz, I., Brillot, R., Lafidil, F., Teixeira-Clerc, F., Nguyen, C. T., et al. (2019). Small-Molecule Inhibitors of Cyclophilins Block Opening of the Mitochondrial Permeability Transition Pore and Protect Mice From Hepatic Ischemia/Reperfusion Injury. Gastroenterology 157, 1368–1382. doi: 10.1053/j.gastro.2019.07.026

Panticak, K. S., Qin, B., and Anderson, R. A. (2015). Ischemia-induced endothelial cell swelling and mitochondrial dysfunction are attenuated by cinnamintannin D1, green tea extract, and resveratrol in vitro. Nutr. Neurosci. 18, 297–304. doi: 10.1179/1476557X14X.661102

Peña-Blanco, A., and García-Sáez, A. J. (2018). Bax, Bak and beyond—sd i s e a s e . Prentice, H., Modi, J. P., and Wu, J. Y. (2015). Mechanisms of Neuronal Protection and Regulation of Mitochondrial Complex I Activity by the Bcl-xL/Bcl-2-Related Factor 2. Cell Death Differ. 12, 713–723. doi: 10.1038/sj.cdd.4401638

Lo et al. Ethnomedicine and MPTP in Apoptosis
Dysfunction in Stroke and Neurodegenerative Diseases. *Oxid. Med. Cell. Longev.* 2015, 964518. doi: 10.1155/2015/964518

Qi, X., Zhou, R., Liu, Y., Wang, J., Zhang, W. N., Tan, H. R., et al. (2016). Trans-cinnamaldehyde protected PC12 cells against oxygen and glucose deprivation/reperfusion (OGD/R)-induced injury via anti-apoptosis and anti-oxidative stress. *Mol. Cell. Biochem.* 421, 67–74. doi: 10.1007/s11010-016-2785-z

Qian, Y., Guan, T., Huang, M., Cao, L., Li, Y., Cheng, H., et al. (2012). Neuroprotection by the soy isoflavone, genistein, via inhibition of mitochondria-dependent apoptosis pathways and reactive oxygen induced NF-κB activation in a cerebral ischemia mouse model. *Neurochem. Int.* 60, 759–767. doi: 10.1016/j.neuroscience.2012.03.011

Ramagiri, S., and Talaty, R. (2016). Neuroprotective effect of hydroxy salfiter yellow A against cerebral ischemia-reperfusion injury in rats: putative role of mPTP. *J. Basic Clin. Physiol. Pharmacol.* 27, 1–8. doi: 10.1515/jbcpp-2015-0021

Reyes-Garcés, N., Diwan, M., Boyaci, E., Gómez-Ríos, G. A., Bojko, B., Nobrega, J. N., et al. (2019). In Vivo Brain Sampling Using a Microextraction Probe Reveals Metabonomic Changes in Rodents after Deep Brain Stimulation. *Anal. Chem.* 91, 9875–9884. doi: 10.1021/acs.analchem.9b01540

Schild, L., Roth, A., Keilhoff, G., Gardemann, A., and Brödemann, R. (2009). Neuroprotection effects of tetrahydroxystilbene glucoside against cerebral ischemia: involvement of JNK, SIRT1, and NF-κB pathways and inhibition of intracellular ROS/RNS generation. *Free Radic. Biol. Med.* 47, 229–240. doi: 10.1016/j.freeradbiomed.2009.02.027

Wang, T., Liu, T., Guan, L., Wang, T., Yuan, X., Zhang, B., et al. (2010). Shikonins protects mouse brain against cerebral ischemia/reperfusion injury through its antioxidant activity. *Eur. J. Pharmacol.* 643, 211–217. doi: 10.1016/j.ejphar.2010.06.027

Wang, H. W., Liou, K. T., Wang, Y. H., Lu, C. K., Lin, Y. L., Lee, I. J., et al. (2011). Deciphering the neuroprotective mechanisms of Bu-yang Huan-wu decoction by an integrative neurofunctional and genomic approach in ischemic stroke mice. *J. Ethnopharmacol.* 138, 22–33. doi: 10.1016/j.ejep.2011.06.033

Wang, T. F., Lei, Z., Li, Y. X., Wang, Y. S., Wang, J., Wang, S. J., et al. (2013). Oxyphorbinidines protects against focal cerebral ischemic injury by inhibiting oxidative stress and apoptosis in mice. *Neurochem. Res.* 38, 2408–2417. doi: 10.1007/s11064-013-1153-6

Wang, H. G., Lan, R., Zhen, X. D., Zhang, W., Xiang, J., and Cai, D. F. (2014). Agong-Niu-Huang Wan protects against cerebral ischemia induced apoptosis in rats: up-regulation of Bcl-2 and down-regulation of Bax and caspase-3. *J. Ethnopharmacol.* 154, 156–162. doi: 10.1016/j.ejep.2014.03.057

Wang, T., Li, Y., Wang, Y., Zhou, R., Ma, L., Hao, Y., et al. (2014). Lycium barbarum polysaccharide prevents focal cerebral ischemic injury by inhibiting neuronal apoptosis in mice. *PloS One* 9, e90780. doi: 10.1371/journal.pone.0090780

Wang, W., Gong, G., Wang, X., Wei-LaPierre, L., Cheng, H., Dirksen, R., et al. (2016). Mitochondrial Flash: Integrative Reactive Oxygen Species and pH Signals in Cell and Organelle Biology. *Antioxid. Redox Signal.* 25, 534–549. doi: 10.1089/ars.2016.6739

Wang, Z., Zhou, Z., Wei, X., Wang, M., Wang, B. O., Zhang, Y., et al. (2017). Therapeutic Potential of Novel Twin Compounds Containing Tetramethylpyrazine and Carnitine Substructures in Experimental Ischemic Stroke. *Oxid. Med. Cell. Longev.* 2017, 7191856. doi: 10.1155/2017/7191856

Wang, H., Chen, Z., Zhang, X., Hu, X., and Sun, H. (2019a). Electroacupuncture ameliorates neuronal injury by PINK1/Parkin-mediated mitophagy clearance in cerebral ischemia/reperfusion. *Nitric. Oxide* 91, 23–34. doi: 10.1016/j. niox.2019.07.004

Wang, H., Wei, W., Lan, X., Liu, N., Li, Y., Ma, H., et al. (2019b). Neuroprotective Effect of Swertiamarin on Cerebral Ischemia/Reperfusion Injury by Inducing the Nrf2 Protective Pathway. *ACS Chem. Neurosci.* 10, 2276–2286. doi: 10.1021/acschemneuro.8b00605
Wu, S., Wu, B., Liu, M., Chen, Z., Wang, W., Anderson, C. S., et al. (2019). Intermodulation Analysis of Huang-Lian-Jie-Du Decoction on Stroke. Front. Pharmacol. 10, 1288. doi: 10.3389/fphar.2019.01288

Wang, W., Hou, Y., Li, Q., Li, X., Wang, W., Ai, X., et al. (2019c). Rhodiola crenulata attenuates apoptosis and mitochondrial energy metabolism disorder in rats with hypobaric hypoxia-induced brain injury by regulating the HIF-1α/microRNA 210/ISCU1/CIIXOX10 signaling pathway. J. Ethnopharmacol. 241, 118101. doi: 10.1016/j.jep.2019.03.028

Wang, X., Liu, Z., Fan, F., Hou, Y., Yang, H., Meng, X., et al. (2019d). Microfluidic chip and its application in autophagy detection. Trends AnaL Tech. 117, 300–315. doi: 10.1016/j.tact.2019.05.043

Wang, Y., Li, B., and Zhang, X. (2019c). Scutellaria baflbata D. Don (SBD) protects brain-derived neurotrophic factor and vascular endothelial growth factor after focal cerebral ischemic infarction in rats. Mol. Biol. Rep. 46, 3817–3826. doi: 10.1007/s11033-019-04824-5

Wang, J. K., Guo, Q., Zhang, X. W., Wang, L. C., Liu, Q., Tu, P. F., et al. (2020). Agha odorata Lour. extract inhibit ischemic neuronal injury potentially via suppressing p53/Puma-mediated mitochondrial apoptosis pathway. J. Ethnopharmacol. 248, 113336. doi: 10.1016/j.jep.2019.113336

Wei, W., Lan, X. B., Liu, N., Yang, J. M., Du, J., Ma, L., et al. (2019e). Echinacoside Alleviates Hypoxic-Ischemic Brain Injury in Neonatal Rat by Enhancing Antioxidant Capacity and Inhibiting Apoptosis. Neurochem. Res. 44, 1582–1592. doi: 10.1007/s11064-019-02782-9

Wei, X., Zhu, Q., Liu, N., Xu, L., Wei, S., Fan, Z., et al. (2019). In Vivo Neuroprotective Effects and Mechanisms of Zhenlong Xingnao Capsule in Vivo and in Vitro Models of Hypoxia. Front. Pharmacol. 10, 1096. doi: 10.3389/fphar.2019.01096

Wei, Y., Zhu, L., Huang, X., and Zhang, Y. (2019). Lipidomics study of protective effect of Salvia miltiorrhiza and Liquisticum chloroform on rats with focal cerebral ischemia injury based on UPLC-Q/TOF-MS. Chin. Traditional Herbal Drugs 50, 408–417. doi: 10.7503/jissn.0253-2670.2019.02.020

Wilson, D. E., Scholl, B., and Fitzpatrick, D. (2018). Differential tuning of excitation and inhibition shapes direction selectivity in ferret visual cortex. Nature 560, 97–101. doi: 10.1038/s41586-018-0354-1

Wu, B., Luo, H., Zhou, X., Cheng, C. Y., Lin, L., Liu, B. L., et al. (2017). Safflower–induced neuronal mitochondrial fission and hexokinase II malfunction in ischemic stroke: Therapeutic effects of kaempferol. Biochim. Biophys. Acta Mol. Basis Dis. 1863, 2307–2318. doi: 10.1016/j.bbadis.2017.06.011

Wu, J. S., Kao, M. H., Tsai, H. D., Cheung, W. M., Chen, J. J., Ong, W. Y., et al. (2014). Potential advantages of Chinese medicine Taohong Siwu Decoction combined with tissue-plasminogen activator for alleviating middle cerebral artery occlusion-induced embolic stroke in rats. Chin. J. Integr. Med. 1–9. doi: 10.1007/s11655-014-1847-x

Yu, Z. H., Cai, M., Xiang, J., Zhang, Z. N., Zhang, J. S., Song, X. L., et al. (2016). PI3K/Akt pathway contributes to neuroprotective effect of Tengxinquan against focal cerebral ischemia and reperfusion injury in rats. J. Ethnopharmacol. 181, 8–19. doi: 10.1016/j.jep.2016.01.028

Zamzami, N., and Kroemer, G. (2001). The mitochondrial in apoptosis how Pandora’s box opens. Nat. Rev. Mol. Cell Biol. 2, 67–71. doi: 10.1038/35048073

Zeng, K., Li, Y., Yang, W., Ge, Y., Xu, L., Ren, T., et al. (2019). Moringa oleifera seed extracts protects against brain damage in both the acute and delayed stages of ischemic stroke. Exp. Gerontol. 122, 99–108. doi: 10.1016/j.exger.2019.04.014

Zhang, S., Qi, Y., Cao, Y., Yang, Y., Zhao, Q., Jing, R., et al. (2017). Protective effect of flavonoid-rich extract from Rosa laevigata Michx on cerebral ischemia–reperfusion injury through suppression of apoptosis and inflammation. Neurochem. Int. 63, 532–533. doi: 10.1016/j.neuint.2013.08.000

Zhang, Q., Bian, H., Li, Y., Guo, L., Tang, Y., and Zhu, H. (2014). Preconditioning with the traditional Chinese medicine Huang-Lian-Jie-Du-Tang initiates HIF-1α-dependent neuroprotection against cerebral ischemia in rats. J. Ethnopharmacol. 154, 443–452. doi: 10.1016/j.jep.2014.04.022

Zhang, Y., Li, H., Huang, M., Chu, K., Xu, W., Zhang, S., et al. (2014). Neuroprotective effects of Gualou Guizhi decoction in vivo and in vitro. J. Ethnopharmacol. 158, 76–84. doi: 10.1016/j.jep.2014.10.020

Zhang, Y., Yan, Y., Cao, Y., Yang, Y., Zhao, Q., Jing, R., et al. (2017). Potential therapeutic and protective effect of curcumin against stroke in the male albino stroke-induced model rats. Life Sci. 183, 45–49. doi: 10.1016/j.lfs.2017.06.023

Zhang, X., Zhang, Y., Tang, S., Yu, L., Zhao, Y., Ren, Q., et al. (2018). Pien-Tze-Huang protects cerebral ischemic injury by inhibiting neuronal apoptosis in acute ischemic stroke rats. J. Ethnopharmacol. 219, 117–125. doi: 10.1016/j.jep.2018.03.018

Zhang, Y. M., Xu, Y. Y., Zhai, J. H., Tao, L. N., Gao, H., Song, Y. Q., et al. (2018). Xingnaojing Injection Protects against Cerebral Ischemia Reperfusion Injury via PI3K/Akt-Mediated eNOS Phosphorylation. Evid. Based Complement. Alternat. Med. 2018, 1–13. doi: 10.1155/2018/2361046

Zhang, H., Xing, Y., Chang, J., Wang, L., An, N., Tian, C., et al. (2019). Efficacy and Safety of NaoShuanTong Capsule in the Treatment of Ischemic Stroke: A Meta-Analysis. Front. Pharmacol. 10, 1133. doi: 10.3389/fphar.2019.01133
Zhang, Q., Liu, J., Zhang, M., Wei, S., Li, R., Gao, Y., et al. (2019). Apoptosis induction of fibroblast-like synoviocytes is an important molecular mechanism for herbal medicine and its active components to treat rheumatoid arthritis. *Biomolecules* **9**, 795. doi: 10.3390/biom9120795

Zhang, T., Hernandez, O., Chrapkiewicz, R., Shai, A., Wagner, M. J., Zhang, Y., et al. (2019). Kilohertz two-photon brain imaging in awake mice. *Nat. Methods* **16**, 1119–1122. doi: 10.1038/s41592-019-0597-2

Zheng, C. Y., Zhang, H. Y., and Tang, X. C. (2008). Huperzine A attenuates mitochondrial dysfunction after middle cerebral artery occlusion in rats. *J. Neurosci. Res.* **86**, 2432–2440. doi: 10.1002/jnr.21681

Zhao, Q., Wang, X., Chen, A., Cheng, X., Zhang, G., Sun, J., et al. (2018b). Rhein protects against cerebral ischemic-/reperfusion-induced oxidative stress and apoptosis in rats. *Int. J. Mol. Med.* **41**, 2802–2812. doi: 10.3892/ijmm.2018.3488

Zhao, B., Sun, L. K., Jiang, X., Zhang, Y., Kang, J., Meng, H., et al. (2019a). Genipin protects against cerebral ischemia-reperfusion injury by regulating the UCP2-SIRT3 signaling pathway. *Eur. J. Pharmacol.* **845**, 56–64. doi: 10.1016/j.ejphar.2018.12.028

Zhao, P., Zhou, R., Li, H. N., Yao, W. X., Qiao, H. Q., Wang, S. J., et al. (2015a). Neuroprotective effects of water-soluble *Ganoderma lucidum* polysaccharides on cerebral ischemic injury in rats. *J. Ethnopharmacol.* **151**, 154–164. doi: 10.1016/j.jep.2015.10.023

Zhu, B., Cao, H., Sun, L., Li, B., Guo, L., Duan, J., et al. (2018). Metabolomics-based mechanisms exploration of Huang-Lian Jie-Du decoction on cerebral ischemia via UPLC-Q-TOF/MS analysis on rat serum. *J. Ethnopharmacol.* **216**, 147–156. doi: 10.1016/j.jep.2018.01.015

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* **94**, 909–950. doi: 10.1152/physrev.00026.2013

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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