

## Review

# Therapeutic effects of herbal compounds in cerebral ischemia with special reference to suppression of microglia activation implicated in neurodegeneration

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**Summary.** Cerebral ischemia affects many especially with the ageing population. The ensuing ischemic reactions include oxidative stress, inflammation, and excitotoxicity among others. In the search for effective therapeutic strategies for cerebral ischemia, activated microglia which are the key player in neuroinflammation are now recognized as a potential therapeutic target. Microglia possess both neurotoxic and neuroprotective roles. They are protective by continuously surveilling the microenvironment, phagocytosing dead cells, secreting trophic factors and sculpting the neuronal connections by removing axons and pruning excess synapses. On the other hand, hyperactivated microglia may impair cerebral oxidative metabolism, and produce excessive proinflammatory mediators that may exacerbate the brain damage. In view of this, suppression of microglial activation has been considered a therapeutic strategy to mitigate microglia-based neuroinflammation in cerebral ischemia. However, balancing the neuroprotective and neurotoxic roles of activated microglia remains a challenging issue. Many traditional Chinese herbal agents have been used in clinic for treatment of cerebral ischemia. Here, we provide an overview of five common Chinese herbs targeting specifically microglia-mediated neuroinflammation in cerebral ischemia. It is hoped that

a common parallel may be drawn from their beneficial effects especially in the latter pathological conditions for their better and effective use in the future.

**Key words:** Cerebral ischemia, Microglia, Neuroinflammation, Herbal agents, Proinflammatory mediators, Signaling pathways

### Introduction

Cerebral ischemia includes ischemic stroke, transient ischemic attack, cerebral arteriosclerosis etc. In addition, neonatal hypoxic ischemic encephalopathy (HIE) is also a common type of cerebral ischemia. In view of its high morbidity and mortality, cerebral ischemia continues to receive wide interest both in basic and clinical studies. Thus, many studies have been carried out in search for effective therapies to ameliorate the injuries resulting from cerebral ischemia such as intravenous thrombolysis with tissue plasminogen activator in 4.5 hours after

**Abbreviation.** AD, Alzheimer's disease; ERK, extracellular signal regulated protein kinases; HO-1, heme oxygenase-1; I/R, ischemia/reperfusion; IFN- $\gamma$ , interferon- $\gamma$ ; IL-1 $\beta$ , Interleukin-1 $\beta$ ; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MAPK, Mitogen-activated protein kinase; MCAO, Middle cerebral artery occlusion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NF- $\kappa$ B, Nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells; NMDA, GluN2A- and GluN2B-containing N-methyl-d-aspartate; NSCs, neural stem cells; NO, nitric oxide; OGD/R, oxygen-glucose deprivation/reperfusion; Prx, peroxiredoxin; ROS, reactive oxygen species; TNF- $\alpha$ , tumor necrosis factor- $\alpha$

stroke (Meurer et al., 2018). Unfortunately, therapeutic time window and treatment expenses have limited the utilization of tissue plasminogen activator. Other options may include antiplatelet therapy and anticoagulant therapy. Therefore, it is imperative to develop alternative and effective treatments for cerebral ischemia. Traditional Chinese herbs have long been used as adjuvant strategy to treat this disease with fewer undesirable side-effects. In this regard, the search for Chinese herbal agents for more effective treatment of cerebral ischemic diseases has attracted the attention of many in recent years.

The developing and mature brain is extremely vulnerable to ischemia and hypoxia because of its great demand for blood, oxygen and energy supply (Thornton and Hagberg, 2015). A sudden disruption to the latter would trigger a multi-faceted cascade of events such as excess release of proinflammatory mediators, free radicals, antioxidant enzymes etc. that may be either neurotoxic or neuroprotective. Brain injury is progressive and can be divided into three phases: the primary energy failure is due to the interruption of blood flow. In hypoxic-ischemia, a sequence of pathological changes is elicited as manifested by an increase in production of reactive oxygen species (ROS), excitatory amino acids, accumulation of free cytosolic calcium, oxidative stress, depletion of ATP-dependent Na/K pump, and mitochondrial function impairment due to deficiency of blood supply and lower levels of oxygen. After a progressive membrane depolarization, excessive excitatory amino acids accumulate in the extracellular spaces along with influx of excessive water, sodium and calcium into the cell. Dysfunction of mitochondria and cellular edema will eventually activate the apoptosis or necrotic signaling pathways. The second phase is reoxygenation and reperfusion of the brain, which is also called "the incubation stage". With the recovery from asphyxiation, partial cerebral blood flow, phosphokinase and ATP function may recover. Cellular edema seems to subside temporarily but energy failure tends to set in again within 6-48 hours. The process of excitability toxicity, oxidative stress and mitochondrial injury continues unabated. Coupled with this is increased expression of proinflammatory mediators leading ultimately to cell death. Next, the proinflammatory response may become chronic triggering a cascade of biochemical reactions. Overactivated microglia, injured neurons and reactive astrocytes all may contribute and exacerbate the brain injury by releasing large amounts of inflammatory mediators or harmful substances. All these may account for the undesirable outcomes, for example, diminished neurogenesis, synaptogenesis and axonal growth so that the brain functions are compromised.

There is mounting evidence pointing to microglia as the key players in neuroinflammation. Once elicited, microglial activation is involved in massive production of a multitude of proinflammatory mediators. Recent studies have shown that Scutellarin, a Chinese herbal compound, effectively attenuated microglia-based

inflammation in experimentally induced cerebral ischemia in rats. Scutellarin also improved the behavioral scores in cerebral ischemic rats as well as promoting microglia-mediated astrogliosis at the infarcted areas and is believed to be beneficial. This has prompted us to review in depth the specific roles of Scutellarin on microglia-mediated neuroinflammation along with other common herbal compounds. It is hoped that some common parallels may be drawn from this with special reference to their effects on microglia activation in cerebral ischemia.

### **Microglia activation and its regulation in cerebral ischemia**

Microglia have been recognized as long-living resident immune cells in the central nervous system (CNS), whose functions are to maintain the chemical and physical microenvironment stability of the brain (Kushchayev et al., 2014; Bruttger et al., 2015). Though microglia constitute only a little part of the total glial population in the brain (5-10%), they exert immense effects in the immune function of the CNS (Paterson et al., 1973; Ling and Wong, 1993). In the acute stage in brain injury, including ischemic injuries (Li et al., 2017a), brain tumors (Menzel et al., 2018), multiple sclerosis (Heidker et al., 2017), Alzheimer's and Parkinson's disease (AD) (Guillot-Sestier and Town, 2018), microglia partake in a protective function by phagocytosing dead cells and degenerating axons (Zelante and Ricciardi-Castagnoli, 2012). In abnormal conditions, ramified microglia are activated and transformed into the amoeboid phenotype whose round cell body or soma shows copious cytoplasm rich in organelles or inclusions. In chronic activation and over a protracted period, activated microglia may produce excess proinflammatory cytokines and/or cytotoxic factors, such as nitric oxide (NO), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and ROS (Vexler et al., 2006; Jin et al., 2010). Microglia are often described to act like a double-edged sword in brain pathologies because they play an important role in clearing cell debris, modulating synaptic plasticity and secreting different trophic factors. In the latter, insulin-like growth factor-1 (IGF-1) and transforming growth factor- $\beta$  (TGF- $\beta$ ) have been shown to promote the survival of neurons and oligodendrocytes. Meanwhile, chronic activation of microglia may result in ever-increasing proinflammatory cytokines, chemokines, ROS and NO that can exacerbate brain injuries (Ransohoff and Perry, 2009; Torres-Platas et al., 2014).

Developmentally, microglia first originate from mesodermal sources during the fetal period (Dalmau et al., 1997). Once settled in the brain parenchyma, and in the mature brain, microglia present a small flattened cell body bearing many long extending and branching processes, and hence are referred to as ramified microglia. Microglia act like a neuropathology sensor because they respond swiftly to any perturbations in the ambient environment by assuming a round or amoeboid

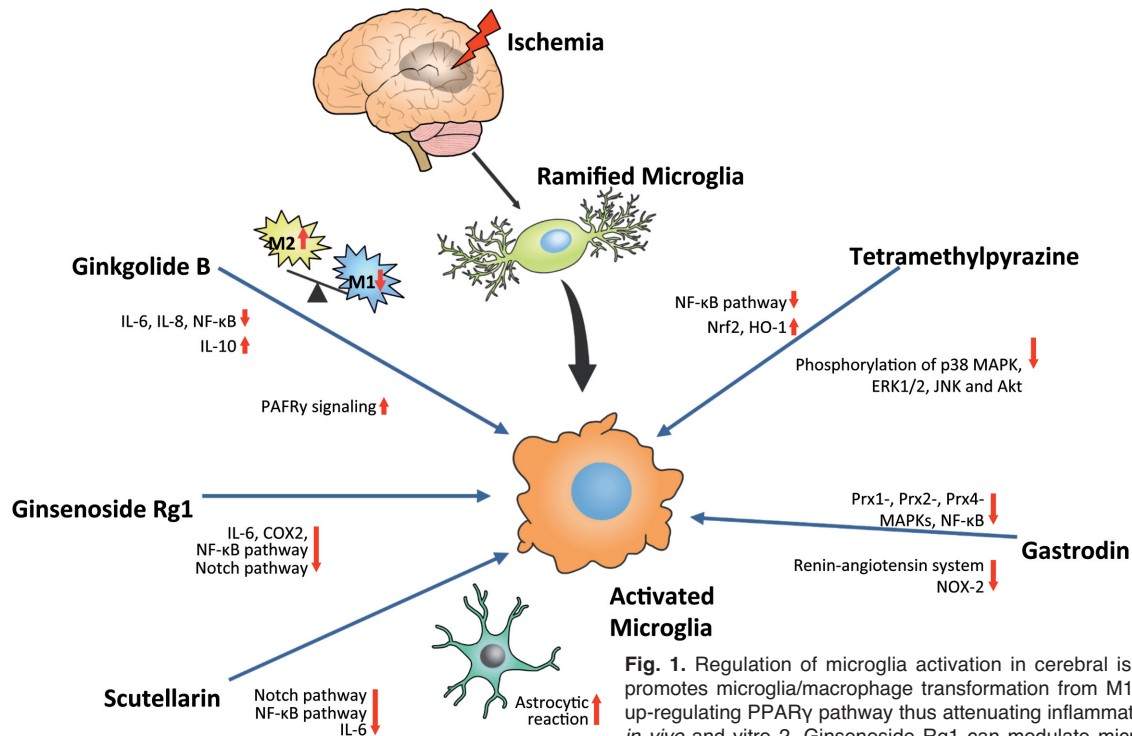
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phenotype. They serve as immunocompetent macrophages in general to maintain the immune balance of brain microenvironment. The beneficial roles of microglia include their involvement in phagocytosis of dead cells/debris as well as maintenance of proper neuronal connections. This is accomplished by pruning excess axons and synapses (Brockhaus et al., 1996; Petersen and Dailey, 2004). Meanwhile, in order to maintain the normal activity and function of neurons and oligodendrocyte, they secrete sufficient neurotrophic factors such as IGF-1 and TGF- $\beta$  among others (Petersen and Dailey, 2004; Deng et al., 2008). Activated microglia can also be counteractive by producing excessive proinflammatory cytokines, chemokines, ROS and NO that may aggravate brain injury (Merrill, 1992).

Recent studies have extended that microglia activation is regulated epigenetically by specific microRNAs in the pathophysiology of cerebral ischemia (Zhao et al., 2013). microRNAs affected the activation or polarization of microglia, and consequently either aggravated or ameliorated microglia-mediated brain

injuries (Karthikeyan et al., 2016; Su et al., 2016). Among the microRNAs that may be involved in regulation of microglia activation include miR-21, miR-124, miR-181c etc., which have been shown to decrease ischemic infarct size, protect damaged neurons, promote neurogenesis, and down-regulate pro-inflammatory mediators (Weng et al., 2011; Zhang et al., 2012, 2015). Among the variety of Chinese herbs, ginkgolide B, ginsenoside, Gastrodin, tetramethylpyrazine and Scutellarin have recently been shown with strong scientific basis to have the property of suppressing microglia activation such as in cerebral ischemic injury (Table 1, Fig. 1). The possibility that the effect of these herbal compounds may act through modulating specific microRNAs in activated microglia has recently been considered (Table 2). However, it remains to be explored the mechanism by which these five common traditional Chinese herbs would regulate microglial activation.

There is growing recognition in recent years of the beneficial values of natural products especially among the Chinese traditional herbs which have been used in



**Fig. 1.** Regulation of microglia activation in cerebral ischemia. 1. Ginkgolide B promotes microglia/macrophage transformation from M1 into M2 phenotype via up-regulating PPAR $\gamma$  pathway thus attenuating inflammatory damage of the brain *in vivo* and *in vitro*. 2. Ginsenoside Rg1 can modulate microglia activation through

NF- $\kappa$ B and Notch pathways. Moreover, Rg1 decreases the expression of IL-6 and COX2. 3. Tetramethylpyrazine (TMP) enhances expression of Nrf2/HO-1 and decreases expression of Phosphorylation of p38 MAPK, ERK1/2, JNK and Akt. TMP confers its neuroprotective function on activated microglia through inhibiting NF- $\kappa$ B signal pathways. 4. Scutellarin exerts its therapeutic effects acting primarily on activated microglia. It attenuates neuroinflammation via suppression of Notch-1 signaling pathway and NF- $\kappa$ B pathway in activated microglia in MCAO rats and in BV-2 microglia. Scutellarin also decreases the expression of IL-6 and promotes microglia-mediated astrogliosis involved in production of neurotrophic factors and tissue repair. 5. Gastrodin has been shown to down-regulate the RAS system, expression of Prx1/2/4, TLR4. It also decreases the expression of MAPKs, NF- $\kappa$ B and NOX-2. All five herbal agents act in common to decrease the production of proinflammatory mediators including iNOS, TNF- $\alpha$ , IL-1 $\beta$ , NO and ROS. In addition, they promote secretion of neurotrophic factors and anti-inflammatory cytokines such as TGF- $\beta$  and IL-10 that may quench microglia-mediated inflammatory response.

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**Table 1.** Anti-inflammatory effects of herbal compounds on activated microglia *in vivo* and/or *in vitro*.

TCMs	Isolated from the herb (Chinese Name)	CNS pathology	Pathway of function examined/identified	Models	References
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	ischemic stroke	cerebral infarction size↓, brain edema↓, behavior↑, memory↑, pro-inflammatory↓, anti-inflammatory cytokines↑/ PPARγ pathway, anti-inflammation	MCAO/R in mice, OGD/R in BV-2 cells	Liu et al., 2018
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	ischemic stroke	M1 phenotype↓, M2 phenotype↑/ neuroprotection	tMCAO on C57BL/6J male mice, LPS induced in cultured BV-2 microglia	Shu et al., 2017
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	multiple sclerosis	PSD95-positive synapse loss↓/ PAFR signaling, anti-inflammation	hippocampal degeneration in C57BL/6 mice	Bellizzi et al., 2016
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	focal cerebral ischemia/reperfusion	TNF-α↓, IL-1β↓, iNOS↓, Bax↑, Bcl-2↓, caspase-3↓/ anti-inflammation and anti-apoptosis	tMCAO/R in mice	Gu et al., 2012
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	acute cerebral inflammatory damage	rough endoplasmic reticulum↑, polyribosomes↑, OX-42 positive microglia↓ / anti-inflammation	LPS intraventricular injection in rat	Liu et al., 2007
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	neurodegenerative diseases	NO↓/ anti-inflammation	LPS-stimulated microglia	Du and Li, 1998a
Ginkgolide B	<i>Ginkgo biloba</i> L. (yinxing)	neurodegenerative diseases	IL-1β↓, NO↓/ anti-inflammation	LPS-stimulated in neonatal rat	Du and Li, 1998a
Ginsenoside Rg1	Ginseng (sanqi)	PD	TNF-α↓, IL-1β↓, α-synuclein↓/ anti-inflammation	MPTP/(MPTP/p) induced in mouse	Heng et al., 2016
Ginsenoside Rg1	Ginseng (sanqi)	stroke	NO↓, cell viability↑, TGF-β1↑, TNF-α↓, / Fcy-receptor-mediated phagocytosis pathway, anti-neurotoxic, neuroprotective	OGD induced in BV-2 cells	Wang et al., 2015a,b
Ginsenoside Rg1	Ginseng (sanqi)	PD	TH-positive cells in the SNpc region↑, CD3(+) CD4(+) to CD3(+)CD8(+) T cells↑, CD4(+) CD25(+)Foxp3(+) regulatory T cells↑, TNF-α↓, IFN-γ↓, IL-1β↓, IL-6↓, / anti-inflammation	MPTP induced in mouse	Sun et al., 2016a
Ginsenoside Rg1	Ginseng (sanqi)	PD	TH positive neurons↑, DA↑, TNF-α↓, IL-1β↓, NO↓, IκB↑, ERK1/2↓, JNK↓, p38 MAPK↓/ anti-inflammation	LPS-induced microglia activation and dopaminergic neuronal degeneration in rat substantia nigra	Zhou et al., 2015
Ginsenoside Rg1	Ginseng (sanqi)	stroke	neuronal cell viability↑, LDH↓, NMDA receptor subunit 1↓, caspase-3↓, mitochondrial transmembrane potential↑, mitochondrial ultrastructure↑/ neuroprotection	OGD in microglia cells, MG-CM in N2a neuronal cells	Wang et al., 2015a,b
Ginsenoside Rg1	Ginseng (sanqi)	neurodegenerative diseases	ROS↑, p-MAPKs↓, p-NF-κB↓, Bcl-2↑, Bax↓, Caspase-3↓, LC3↓, Beclin-1↓ / anti-oxidation	t-BHP-mediated cell damage in BV-2 microglial cells	Lu et al., 2015
Ginsenoside Rg1	Ginseng (sanqi)	ischemia	NO↓, TGF-β1, TNF-α↓/ anti-inflammation, balance microglia homeostasis	OGD in microglia cells	Wang et al., 2014b
Ginsenoside Rg1	Ginseng (sanqi)	neuropsychiatric disorders	weight loss↓, anorexic- and depressive-like behavior↑, pro-inflammatory mediators↓, neurotoxic species↓/anti-neuroinflammation	LPS-induced in rats	Zheng et al., 2014
Ginsenoside Rg1	Ginseng (sanqi)	neurodegenerative diseases	phospho-p38↑, iNOS↑, COX2↑/ anti-neuroinflammation	LPS-induced in microglial cells	Lee et al., 2012a,b
Ginsenoside Rg1	Ginseng (sanqi)	neurodegenerative diseases	iNOS↓, COX-2↓, TNF-α↓, IL-1β↓, NF-κB↓/ PLC-γ1 signaling pathway, anti-inflammation	LPS-stimulated in murine BV-2 microglial cells	Zong et al., 2012
Ginsenoside Rg1	Ginseng (sanqi)	neurodegenerative diseases	NO↓, TNF-α↓, Iba-1↓, iNOS↓, NFκB and MAPK pathway↓/ anti-inflammation	LPS-induced in microglial cells	Hu et al., 2011
Ginsenoside Rg1	Ginseng (sanqi)	neurodegenerative diseases	NO↓, TNF-α↓, JNK↓, ERK↓/ anti-inflammation	LPS-activated in N9 microglial cells	Wu et al., 2007
Ginsenoside Rg1	Ginseng (sanqi)	neurodegenerative diseases	TNF-α↓, IL-6↓, IL-1β↓, bcl-2↑, bax↓/ anti-apoptosis, anti-inflammation	chronic brain inflammation in rats	Joo et al., 2005
Ginsenoside Rg1	Ginseng (sanqi)	AD	NO↓/ anti-oxidation, anti-inflammation	Abeta25-35 and/or IFN-γ induced in microglia	Gong et al., 1999
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	Traumatic brain injury	periorbital hypersensitivity↓, SP↑, iNOS↓, CGRP↑, IL-6↓, TNF-α↓, IL-12↓/ anti-neuroinflammation	TBI model in mouse	Wang et al., 2017c
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	permanent cerebral ischemia	neuronal loss↓, macrophage/microglia activation↓, brain parenchyma infiltrative neutrophils↑, circulating neutrophils↑, endothelium adhesion↑, NO↓, HMGB1↓, TLR4↑, Akt↓, ERK↓, iNOS↓, Nr2↑, HO-1↑/ anti-inflammation, promote defense capacity	permanent cerebral ischemia in rats	Chang et al., 2015
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	neurodegenerative diseases	iNOS↓, ERO1L↓/ anti-neuroinflammation	LPS-induced in N9 microglial cell	Pu et al., 2015

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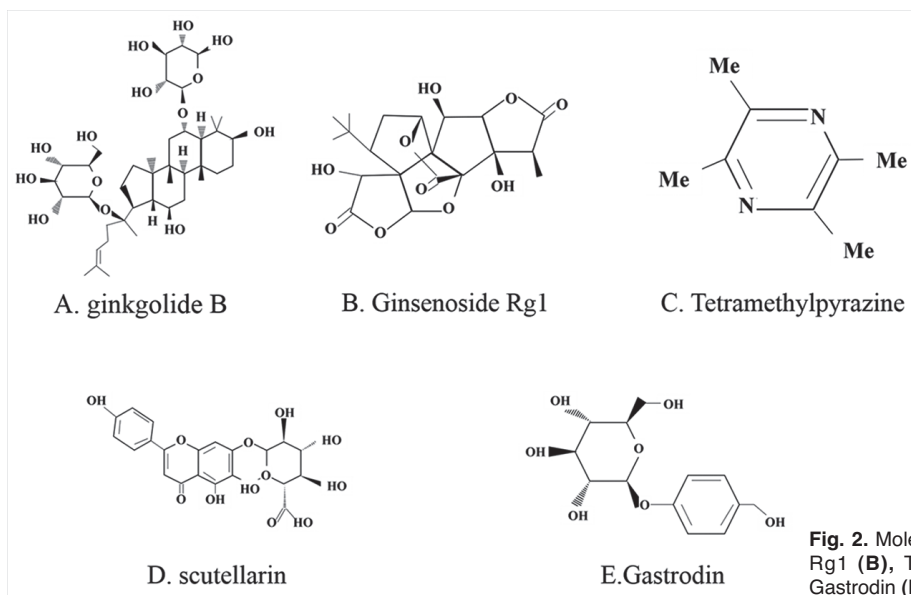
Table 1. (Continuation).

Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	AD	NO $\downarrow$ TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , MCP-1 $\downarrow$ , iNOS $\downarrow$ , phosphorylation of Akt $\downarrow$ , neuronal death $\downarrow$ / neuroprotection	organotypic hippocampal slice cultures, A $\beta$ 25-35 stimulated in cultured microglial cells	Kim et al., 2014b
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	spinal cord injury	TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , COX-2, microglia activation $\downarrow$ , neutrophil infiltration $\downarrow$ , / modulatory role in microglia activation, neuroprotection	spinal cord compression injury in mice	Shin et al., 2013
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	ischemic stroke	percentages of activated macrophages/ microglia and infiltrative lymphocytes $\downarrow$ , neutrophils $\downarrow$ , macrophages $\downarrow$ , pro-inflammatory cytokine $\downarrow$ , Nrf2 $\uparrow$ , HO-1 $\uparrow$ / anti-inflammation, neuroprotection	permanent cerebral ischemia in rats	Kao et al., 2013
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	neurodegenerative diseases	NO $\downarrow$ , iNOS $\downarrow$ , NF- $\kappa$ B nuclear translocation $\downarrow$ phosphorylation of p38 MAPK, ERK1/2, JNK and Akt $\downarrow$ ROS $\downarrow$ / anti-inflammation, anti-neurotoxic factors	LPS-induced in N9 microglial cells	Liu et al., 2010
Tetramethylpyrazine	Ligusticum chuanxiong hort (chuanxiong)	ischemic deficits	neuronal loss $\downarrow$ , brain infarction $\downarrow$ , Bcl-xL $\uparrow$ MCP-1 $\downarrow$ / anti-inflammation, anti-apoptosis, neuroprotection	focal cerebral I/R by CCA and MCAO model in rats	Kao et al., 2005
Scutellarin	Breviscapine (dengzhanhuasu)	neurodegenerative and cerebrovascular diseases	TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , IL-6 $\downarrow$ , NO $\downarrow$ , phosphorylation of NF- $\kappa$ B-p65, p38, JNK, and AKT $\downarrow$ / IKK-dependent NF- $\kappa$ B and p38/JNK signaling pathway, anti-inflammation	LPS-induced in BV-2 cells	You et al., 2018
Scutellarin	Breviscapine (dengzhanhuasu)	cerebral ischemia	microglial activation $\downarrow$ , astrocytic reaction $\uparrow$ , neurotrophic factors $\uparrow$ / anti-neuroinflammation, neuroprotection	MCAO in rats, LPS in BV-2 cells	Wu et al., 2017a
Scutellarin	Breviscapine (dengzhanhuasu)	cerebral ischemia	GFAP $\uparrow$ , MAP-2 $\uparrow$ , BDNF $\uparrow$ , NT-3 $\uparrow$ , IGF-1 $\uparrow$ , neurological scores $\uparrow$ , infarct size $\downarrow$ / neuroprotection, facilitates tissue remodeling	MCAO in rats, conditioned medium from BV-2 microglia in TNC 1 and primary astrocytes	Fang et al., 2016
Scutellarin	Breviscapine (dengzhanhuasu)	cerebral ischemia	GFAP $\uparrow$ , Notch-1 $\uparrow$ , NICD $\uparrow$ , HES-1 $\uparrow$ , TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , iNOS $\downarrow$ , astrocytic reaction $\uparrow$ / neuroprotection	MCAO in rats, LPS-activated BV-2 cells in TNC1 astrocytes	Fang et al., 2015a
Scutellarin	Breviscapine (dengzhanhuasu)	ischemic stroke	NF- $\kappa$ B $\downarrow$ , Notch-1 $\downarrow$ , NICD $\downarrow$ , RBP-JK $\downarrow$ , Hes-1 $\downarrow$ , MCP-1 $\downarrow$ , microglial migration $\downarrow$ , microglia adhesion $\uparrow$ , cytoskeleton of microglia $\uparrow$ / anti-neuroinflammation	MCAO in rats, LPS in BV-2 cells	Yuan et al., 2015
Scutellarin	Breviscapine (dengzhanhuasu)	ischemic stroke	infarct size $\downarrow$ , TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , iNOS $\downarrow$ , NO $\downarrow$ , ROS $\downarrow$ /anti-inflammation	MCAO in rats, LPS in BV-2 cells	Yuan et al., 2014
Scutellarin	Breviscapine (dengzhanhuasu)	hypertension-associated diseases	TLR4 $\downarrow$ , NF- $\kappa$ B p65 $\downarrow$ , TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , IL-18 $\downarrow$ , Bax $\downarrow$ , cleaved-caspase-3 p17 $\downarrow$ , Mcl1 $\uparrow$ / anti-inflammation, anti-apoptosis	renovascular hypertensive in rats	Chen et al., 2013
Scutellarin	Breviscapine (dengzhanhuasu)	neurodegenerative diseases and stroke	NO $\downarrow$ , TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , ROS $\downarrow$ , iNOS $\downarrow$ , NF- $\kappa$ B $\downarrow$ , JNK and p38 phosphorylation $\downarrow$ / anti-inflammation	LPS-induced in BV-2 cells	Wang et al., 2011
Gastrodin	Gastrodia elata Blume (tianma)	ischemic-hypoxia	RAS $\downarrow$ , proinflammatory mediators $\downarrow$ , Sirt3 $\uparrow$ , NOX-2 $\downarrow$ /anti-inflammation	HIBD, LPS in BV-2	Li et al., 2018
Gastrodin	Gastrodia elata Blume (tianma)	ischemic brain injury	Prx1/2/4 $\downarrow$ , TLR4 signaling activation $\downarrow$ , inflammatory mediator production $\downarrow$ /anti-inflammation	MCAO in rats	Mao et al., 2017
Gastrodin	Gastrodia elata Blume (tianma)	acute ocular hypertension	TNF- $\alpha$ $\downarrow$ , iNOS $\downarrow$ , phosphorylated p38 MAPK $\downarrow$ /anti-inflammation	acute ocular hypertension (AOH) rat	Wang et al., 2017a
Gastrodin	Gastrodia elata Blume (tianma)	chronic inflammatory pain	AMPA receptors $\downarrow$ , NMDA receptors $\downarrow$ , CaMKII- $\alpha$ $\downarrow$ , TNF- $\alpha$ and IL-6 $\downarrow$ /anxiolytic, /anti-inflammation	chronic inflammatory pain	Sun et al., 2016b
Gastrodin	Gastrodia elata Blume (tianma)	AD	memory impairments $\uparrow$ . A $\beta$ deposition $\downarrow$ , glial activation $\downarrow$ , /anti-inflammation, anti-amyloidogenic effects	AD in Tg2576 mice	Hu et al., 2014
Gastrodin	Gastrodia elata Blume (tianma)	PD	moving latency $\uparrow$ , tyrosine hydroxylase-positive cells $\uparrow$ , IL-1 $\beta$ $\downarrow$ , /anti-inflammation	rotenone-induced Parkinson's disease model in rats	Li et al., 2012
Gastrodin	Gastrodia elata Blume (tianma)	AD	CHOP $\downarrow$ , GRP78 $\uparrow$ /anti-apoptosis	$\beta$ -amyloid-induced in BV-2 mouse microglial cells	Lee et al., 2012a,b
Gastrodin	Gastrodia elata Blume (tianma)	neurodegenerative diseases	iNOS $\downarrow$ , COX-2 $\downarrow$ , TNF- $\alpha$ $\downarrow$ , IL-1 $\beta$ $\downarrow$ , NF- $\kappa$ B $\downarrow$ , ERK1/2 $\downarrow$ , JNK $\downarrow$ , p38 MAPK $\downarrow$ , /anti-neurotoxicity, anti-inflammation	LPS-stimulated in cultured murine microglial BV-2 cells	Dai et al., 2011
Gastrodin	Gastrodia elata Blume (tianma)	nerve cells apoptosis and decrease learning capacity of diabetics	IL-1 $\beta$ $\downarrow$ , IL-6 $\downarrow$ /anti-apoptosis, neuroprotection	high concentration of glucose in BV-2 cells	Du et al., 2009

clinic, and health diets among others (Atanasov et al., 2015). In light of this, the bioactive compounds in them have drawn wide interest especially on their therapeutic potential and the underlying molecular mechanisms of action (Table 3) (Kim et al., 2014a). Extensive research on natural products may lead to drug discovery (Bauer and Bronstrup, 2014). In view of the detrimental sequelae in microglia-mediated neuroinflammation, it is therefore desirable to have a fuller understanding of the effects of various herbal compounds targeting specifically the activated microglia. This review will focus on the effects of five common Chinese herbs, namely, ginkgolide B, ginsenoside, Gastrodin, tetramethylpyrazine and Scutellarin on microglia-mediated neuroinflammation with special reference to the cerebral ischemia.

### Effects of ginkgolide B on microglial activation in cerebral ischemia

*Ginkgo biloba*, a native ancient traditional Chinese herb, has been well documented for thousands of years for its wide distribution. It grows slowly for more than twenty years to ripe. Both leaves and seeds of *Ginkgo biloba* can be used for medical purposes. Known as a strong anti-platelet activating factor (PAF), it possesses antioxidant stress and anti-apoptosis properties. *Ginkgo biloba* and its bioactive compounds, such as Yinxingdamo (YXDM) and *Ginkgo biloba* EGb 761, are now widely used for treatment of coronary heart disease (Wang et al., 2014c), ischemic stroke (Luo et al., 2017), cognitive function (Li et al., 2017b), and cancer (Liu et al., 2017). Enriched with biflavones, *Ginkgo biloba* can



**Fig. 2.** Molecular structure of Ginkgolide B (A), Ginsenoside Rg1 (B), Tetramethylpyrazine (C), Scutellarin (D) and Gastrodin (E).

**Table 2.** miRNAs and their targets involved in cerebral ischemia.

TCMs	Cell Type	MicroRNA implicated	Targets (mRNA)	Role in inflammation	References
Scutellarin	BV-2	miR-181a	TNF- $\alpha$	Inhibits microglia activation	unpublished data
Ginkgolide	-	-	-	-	-
	mADSC	miRNA-124	small C-terminal domain phosphatase 1 (SCP1)	promotes the differentiation of mouse adipose-derived stem cells (mADSC) towards the neuronal lineage	Dong et al., 2017
Ginsenoside Rg1	HUVEC	miRNA-124	eNOS	promotes angiogenesis	Chan et al., 2009
	endothelial cells	miR-23a	hepatocyte growth factor receptor (MET)	promotes angiogenesis	Kwok et al., 2015
	VEGFR-2	miR-15b	vascular endothelial growth factor receptor-2	promotes angiogenesis	Chan et al., 2013
Tetramethylpyrazine	-	-	-	-	-
Gastrodin	-	-	-	-	-

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Table 3. Signal pathways involved in cerebral ischemia.

TCMs	Isolated from the herb (Chinese name)	Pathway	Pathway of Function Examined/Identified	Models	References
Scutellarin	Breviscapine	Notch pathway	NF- $\kappa$ B/ Notch-1/ NICD/ RBP-JK /Hes-1/ microglia adhesion $\uparrow$ , MCP-1/ microglial migration $\downarrow$ , altered the phenotype of microglia	MCAO in rats, lipopolysaccharide (LPS)-induced BV-2 microglia	Yuan et al., 2015
		PPAR $\gamma$ pathway	cerebral infarction size/brain edema $\downarrow$ , behavior/memory recover $\uparrow$ , regulating balance of pro-/anti-inflammatory microglia polarization	MCAO/reperfusion in mice, OGD/R in BV2	Liu et al., 2018
		pentose phosphate pathway	reverse the I/R induced brain metabolic deviations	tMCAO in mice	Geng et al., 2017
		PI3K pathway	neuronal survival $\uparrow$ , LDH release/ RTP801 mRNA and protein $\downarrow$	OGD in primary cultured cortical neurons	Wu et al., 2015
Ginkgolide. B	Ginkgo biloba L.	JNK signaling pathway	neuronal apoptosis/ p-SAPK/JNK levels/ nuclear translocation/ ROS $\downarrow$ , Bax $\downarrow$ , Bcl-2/ cytochrome-c/ caspases-3 and 9/ PARP $\downarrow$	pMCAO in SD rats	Wang et al., 2014d
		collapsin response mediator protein 2 (CRMP2) pathway	infarct volumes/ neurologic deficits $\downarrow$ , NMDA-induced excitotoxicity $\downarrow$ , Nrf2 /HO1/ GAPDH/ CRMP2/ histone H3 during t-BuOOH-induced oxidative stress $\uparrow$	tMCAO/R in mice, t-BuOOH/ H <sub>2</sub> O <sub>2</sub> /NMDA in primary neurons	Nada et al., 2012
		PI3K signalling pathway	cell viability/ HIF-1 $\alpha$ / EPO/ phosphorylated 136p-Bad/ p-GSK-3 $\beta$ $\uparrow$ , apoptotic cells / caspase-3 $\downarrow$	ischaemia in cultured mouse cortical neurons	Wu et al., 2009
		NIK-IKK pathway	p-NIK/ p-IKK $\alpha$ $\downarrow$ , I $\kappa$ B $\alpha$ / nuclear translocation of NF- $\kappa$ B p65/ NF- $\kappa$ B target gene c-Myc mRNA $\uparrow$	pMCAO in rats	Wang et al., 2008
		TLR2/4 signaling pathways	cell viability/ TLR2/ TLR4/ MyD88/ Bak/ RIP3 $\uparrow$ , IL-1 $\beta$ / IL-6/ IL-8/ IL-10/ TNF- $\alpha$ $\downarrow$ , p-TAK1/ p-I $\kappa$ B $\alpha$ / p-IKK $\beta$ / transfer of NF- $\kappa$ B p65 from cytoplasm to nucleus $\downarrow$	OGD/R in BV-2 cells	Zhou et al., 2016
		HIF-1 $\alpha$ signaling pathway	neurological impairment/ pathologic damage $\downarrow$	neonatal HI model	Tang et al., 2017
Ginsenoside Rg-1	Ginseng	NgR1/RhoA/ ROCK2 pathway	NgR1/ RhoA/ ROCK2 $\downarrow$	MCAO/R in SD rats, OGD/R in SH-SY5Y cells	Shi et al., 2016
		NF- $\kappa$ B pathway, oxidative stress pathway and cytokine network pathway, Nrf2/HO-1 pathway	phosphorylation of NF- $\kappa$ B, p50, p65 and IKK $\alpha$ / $\beta$ in TNF- $\alpha$ -treated EA $\downarrow$	H <sub>2</sub> O <sub>2</sub> -treated PC12 cells	Li et al., 2015
		PPAR $\gamma$ /HO-1 signaling	neurological deficits, apoptosis and inflammation in hippocampus $\downarrow$	MCAO/R in rats	Yang et al., 2015
		PI3K/Akt and ERK1/2 pathways	infarct size and neurological scores $\downarrow$ , Akt phosphorylation/ number of BrdU/DCX and Nestin/ GFAP double-positive cells $\uparrow$ , VEGF and BDNF $\uparrow$	tMCAO in SD rats, OGD/R in PC12 cells	Liu et al., 2015
		PI3K/Akt pathway	neuronal death and mitigated OGD-induced autophagic vacuoles $\downarrow$ , phosphor Akt at Ser473 $\uparrow$	two vessels occlusion in rats, OGD in SH-SY5Y cells	Luo et al., 2014
Tetramethylpyrazine chuanxiong hort	Ligusticum	AMPK/mTOR and JNK pathways	cerebral blood flow $\uparrow$ , infarct volume, brain water content, and the neurological deficits $\downarrow$ , beclin1 and microtubule-associated protein 1 light chain 3 $\downarrow$ , phosphorylation of AMPK and mTOR/ JNK $\downarrow$	MCAO/R in C57BL/6 mice	Guo et al., 2014
		NF- $\kappa$ B signaling pathway	cytotoxicity/ nuclear translocation of NF- $\kappa$ B/p65 $\downarrow$ , phosphorylation of I $\kappa$ B/ IKK/ activation of Akt/ ERK1/2 $\downarrow$	H <sub>2</sub> O <sub>2</sub> -induced PC12 cells	Liu et al., 2011
		HIF-1 $\alpha$ signal pathway	Longa score/ number of apoptotic cells $\downarrow$ , HIF-1 $\alpha$ / CC3 $\uparrow$	HIBD in 10-day-old SD rats	Wang et al., 2010
		JNK signal pathway	apoptosis rates/ JNK kinase MKK4mRNA and MKK7mRNA/ C-fos/ C-jun/ P-JNK $\downarrow$	anoxia/reoxygenation (A/R) rat hippocampal neurons	Zhong et al., 2016
		PI3K pathway	expression and secretion of SDF-1/ PI3K/ Akt/ PKC/ ERK $\uparrow$ , enhanced NPC migration	MCAO in rats	Kong et al., 2016
Gastrodin	Gastrodia elata Blume	HMGB1/TLR4, Akt, and ERK signaling	including HMGB1/ TLR4/ Ak/ ERK, inducible nitric oxide synthase, Nrf2/ HO-1 $\uparrow$	pMCAO in rats	Chang et al., 2015
		PI3K/Akt signaling	NO/ phosphorylation of Akt $\uparrow$	OGD in primary cultured HAECs, MCAO in Wistar rats	Yan et al., 2015
		PI3K/Akt/GSK3 $\beta$ pathway	number of apoptotic cells/ CC3 and Bax $\downarrow$ , Bcl-2/ phosphorylation of Akt and GSK3 $\beta$ $\uparrow$	tMCAO/R in Wistar rats	Chen et al., 2014a
		-	-	-	-

be isolated into different constituents, including terpene trilactones (ginkgolides A, B, C, J, P, and Q, and bilobalides), catechins, proanthocyanins, sterol, and 6-hydroxykynurenic acid. Though it is hard for ginkgolides to gain access into the brain tissue via the blood-brain barrier (BBB), ginkgolides and their bioactive components are modified for usage in clinic. Ginkgolide B, a specific pharmacological diterpenes which isolated from *Ginkgo biloba* (Woelkart et al., 2010) has attracted special interest for its effects in modulating inflammatory oxidative stress, inflammatory and endothelial function not only in ischemic stroke, but also in hemorrhagic stroke (Nabavi et al., 2015).

Ginkgolide B (Fig. 2A) has been identified to decrease ischemic duration and diminish the release of lactate dehydrogenase in neurons by inhibiting the stress-related protein RTP801. Inhibitor of phosphatidylinositol 3-kinase (PI3K) could block such a beneficial effect. Thus, it is suggested that Ginkgolide B may play a neuroprotective role though PI3K pathways in oxygen-glucose deprivation (OGD) injury (Wu et al., 2015). An additional pathway, c-Jun N-terminal kinase (JNK)1/2 /p38 Mitogen-activated protein kinase (MAPK) signaling pathway, has been reported to be neuroprotective through decreasing pro-inflammatory mediators and eliminating free radicals on oxygen-glucose deprivation and reoxygenation (OGD/R) injury (Jiang et al., 2014). 10-O-(N,N-dimethylaminoethyl)-Ginkgolide B methane sulfonate (XQ-1H), a derivative of Ginkgolide B, protected the BBB integrity from disruption and damage by ischemic stroke in hyperlipidemic rat due to over production of proinflammatory cytokines in the rat brain vascular endothelial cells stimulated by LPS (Fang et al., 2015b). In addition to modulating neutrophil infiltration, XQ-1H showed anti-oxidative effects to suppress superoxide dismutase activity and lipid peroxidation in cortical neurons (Wei et al., 2013). Ginkgolides A and B (GKAB) in combination were capable of reducing neuronal apoptosis and several mitochondrial proapoptotic molecules (Wang et al., 2014d). Ginkgolide B was found to reduce the ROS and malondialdehyde levels in hyperglycemia ischemic rats (Huang et al., 2012). It attenuates the pathological damage and extenuates brain edema, inhibits excitatory amino acids and maintains BBB integrity in middle cerebral artery occlusion (MCAO) rat model (Lv et al., 2011; Yang et al., 2011). Moreover, Ginkgolide B alleviated necrotic and apoptotic cell death from ischemia/reperfusion (I/R) injury. It exerted the effect through decreasing the expression and enzymatic activity of cathepsins B and L (Qin et al., 2014).

Two major ingredients of *Ginkgo biloba* leaves, Ginkgolide and bilobalide, have been reported to confer a neuroprotective effect through acting on activated microglia. They reversed the expression of IL-1 $\beta$ , IL-6, IL-8, IL-10, TNF- $\alpha$  and Nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells (NF- $\kappa$ B) p65 nuclear translocation in activated BV-2 microglia induced by

OGD/R. In this connection, Ginkgolide and bilobalide exert anti-inflammatory and neuroprotective effects by regulating TLR2/4 signaling pathways and TLRs/MyD88/NF- $\kappa$ B signaling pathways (Zhou et al., 2016). The same pathway was involved in anti-inflammation and anti-apoptosis with Ginkgolide B in activated microglia. Ginkgolide B could reduce TNF- $\alpha$ , IL-1 $\beta$  and inducible nitric oxide synthase (iNOS), while NF- $\kappa$ B revertant gene p53 and Bax were up-regulated (Gu et al., 2012). In light of the above, Ginkgolide B has great potential for treating ischemic stroke. Remarkably, it promoted microglia/macrophage transformation into M2 phenotype *in vivo* and *in vitro*. More importantly, it decreases the cerebral ischemic damage and neurological deficits from polarization to M1 phenotype (Shu et al., 2016). Concomitantly, it suppresses over-production of inflammatory mediators and apoptosis by inhibiting NF- $\kappa$ B activation in transient MCAO (tMCAO) rats (Gu et al., 2012). Of note, XQ-1H could balance microglia polarization between proinflammatory phenotype and anti-inflammatory phenotype. During this process, peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) mobilization from the nucleus to cytoplasm was increased and was accompanied by decreased neurological deficit from OGD/R injury (Liu et al., 2018).

There is mounting evidence showing the inhibitory properties of Ginkgolide B by decreasing the expression of NO, TNF- $\alpha$ , and IL-1 $\beta$  in LPS-stimulated neonatal rat microglia (Du and Li, 1998a,b). Ginkgolide B (BN52021) effectively reduces neuroinflammation thus improving the cognitive functions of rats in Morris water maze; it decreases OX-42 positive microglia (Liu et al., 2007). A recent study reported that neurons may be injured at their synaptic connections in a mouse model of multiple sclerosis. It was suggested that usage of anti-platelet-activating factor, such as Ginkgolide B, might mitigate the microglia mediated damage to excitatory synapses in the hippocampus (Bellizzi et al., 2016). Furthermore, a mixture containing Ginkgolide B might be a potential therapeutic option for prion or Alzheimer's disease (AD) as a platelet-activating factor antagonist. Pretreatment with this compound would save SH-SY5Y neuroblastoma cells from killing by microglia stimulated degeneration of amyloid-beta1-42 or sPrP106.

### Effects of ginsenoside Rg1 on microglial activation in cerebral ischemia

Ginseng, as a traditional herbal medicine, has been extensively used in China, Korea, and Japan for over 2000 years. Although *Panax ginseng*, *Panax quinquefolium*, and *Panax notoginseng* belong to the same genera of Ginseng family, each is grown in different areas and is endowed with special features in biology. *Panax ginseng* adapts to the ecological environment of the north, while *Panax notoginseng* is mainly confined to Yunnan and Guangxi provinces of China. The three herbs share the same bioactive



ingredients, such as ginsenoside- Rg1, Rg2, Re, Rb1, Rb2 etc. and are widely used in clinical medicine. Moreover, given the exclusive saponins components (Notoginsenoside- R1, R2, R4, R6, Fa) *Panax notoginseng* plays an important role in treatment of cardiac dysfunction (Sun et al., 2013), ischemic stroke (Sozmen et al., 2016), hemorrhagic disorders (Liu et al., 2014), diabetic kidney disease (Gui et al., 2014), and acute liver failure (Zhao et al., 2014). *Panax quinquefolium* is found to contain a certain amount of pseudo-ginsenoside 20 (R) -F11 and ginsenoside RAO.

In recent years, ginsenoside Rg1 (Fig. 2B), the major active ingredient of ginseng, is reported to have various therapeutic actions, especially on the nervous and cardiovascular systems (Deng et al., 2015; Lin et al., 2015). It is well documented that two hallmark pathological features of cerebral ischemia-reperfusion injury are inflammation and neuronal apoptosis. Rg1 has been found to promote neurogenesis by increasing Akt phosphorylation and VEGF and BDNF expression both *in vitro* and *in vivo* through PI3K/Akt and extracellular signal regulated protein kinases (ERK1)/2 pathways (Liu et al., 2015). Similar results were obtained by suppression of inflammation and neuron apoptosis by activating PPAR $\gamma$ / heme oxygenase-1 (HO-1) (Yang et al., 2015) and down-regulation of NF- $\kappa$ B signaling pathway (Liu et al., 2011) with Rg1. Cell injury was thus rescued and oxidative stress alleviated. It is relevant to note that PI3 kinase inhibitor LY294002 could block both of them.

It is known that transient global ischemia would lead to lethal autophagy, and in this regard ginsenoside is used to mitigate I/R-induced autophagic neuronal death in rats and OGD-induced autophagic vacuoles in SH-SY5Y cells (Luo et al., 2014). In clinic, its compounds were explored to inhibit autophagy, enhance cerebral blood flow, and limit cerebral ischemia-reperfusion injury through AMPK/mTOR and JNK pathways (Guo et al., 2014). Apart from its protective effects by decreasing the infarct size and improving the neurological scores in ischemia-reperfusion injury, ginsenoside Rg1 promotes angiogenesis. Rg1 can act as an angiogenesis-inducing compound in different models. miRNA microarray analysis has demonstrated that Rg1 may induce angiogenesis by down- or up-regulated miRNAs (including miR-23a, miR-15b, -214, and -377) in human umbilical vein endothelial cells. Ginsenoside-Rg1-induced angiogenesis may help increase the vascular endothelial growth factor receptor-2 (VEGFR-2) expression and HUVECs migration and tubulogenesis followed by reduced miR-15b expression (Chan et al., 2013). In the same model, Rg1 promoted angiogenesis by down-regulating miR-214 expression, leading to an increase in eNOS expression, as well as an increase of cell migration and tube formation.

As mentioned previously, hyperactivated microglia contribute to several neurological disorders and neurodegenerative diseases. In these, activated microglia is related to oxidative stress, glutamate excitotoxicity,

apoptosis, necrosis and production of excessive inflammatory mediators (TNF- $\alpha$ , IL-1 $\beta$ , ROS and NO). Remarkably, ginseng neutralizes many of the neurotoxic effects detrimental to neurons both *in vivo* and *in vitro*. It drastically decreased the production of these proinflammatory mediators stimulated by LPS. Interestingly, such efficacy seems to be partially blocked by a specific phospholipase C (PLC) inhibitor, U73122 via PLC- $\gamma$ 1 signaling pathway (Zong et al., 2012). It is well documented that neuroinflammation mediated by activated microglia plays a vital role in neurodegenerative diseases, including PD, AD and amyotrophic lateral sclerosis (Lee et al., 2012a,b). Oral treatment with Rg1 has been reported to decrease the release of TNF- $\alpha$  and IL-1 $\beta$  while protecting dopaminergic neurons from abnormal  $\alpha$ -synuclein-mediated neuroinflammation in the mouse SNpc stimulated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/probenecid (MPTP/p) (Heng et al., 2016). In addition, Rg1 reduced the effect of hyperactive microglia by inhibiting over-producing proinflammatory mediators and restraining infiltration of CD3(+) T cells to protect MPTP-induced mice from PD (Zhou et al., 2015). Additionally, Rg1 promoted survival of neurons. It therefore cut down the leakage of LDH, enhanced mitochondrial transmembrane potential and mitochondrial ultrastructure. In the study by Wang et al (Wang et al., 2015b), Rg1 was used to mitigate the OGD damage in microglial cells through decreasing proinflammatory mediators, such as NO and TNF- $\alpha$ , as well as increasing the expression of TGF- $\beta$ . It exerts an effective role in balancing microglia homeostasis after ischemia injuries (Wang et al., 2014b). Ginsenoside Rg1 combined with geniposide can significantly inhibit NO level, protect cell viability, improve the expression of TGF- $\beta$ 1, and reduce the expression of TNF- $\alpha$ . Geniposide in combination with ginsenoside Rg1 may achieve a better equilibrium between TNF- $\alpha$  and TGF- $\beta$ 1 though Fc $\gamma$ -receptor-mediated phagocytosis pathway (Wang et al., 2015a). Ginsenoside Rh2 markedly inhibits microglia activation resulting in decreased generation of NO production, TNF- $\alpha$ , IL-6, IL-1 $\beta$ , cyclooxygenase-2 and iNOS in LPS-induced activated microglial cells. Moreover, Ginsenoside Rh2 increased the expression of TGF- $\beta$ 1 and reduced the expression of Smad. It is suggested that ginsenoside Rh2 plays its role in microglia activation via modulating the TGF- $\beta$ 1/Smad pathway (Vinoth Kumar et al., 2016). Some studies, however, have pointed out that the immunomodulatory and neuroprotective effects may rely on the whole botany, rather than a single ginsenoside (Rb1, Rg1, or Re) (Beamer and Shepherd, 2012).

#### Effects of tetramethylpyrazine on microglial activation in cerebral ischemia

*Ligusticum wallichii* (Chuan Xiong) is widely used in China for its strong pharmacological action and antioxidant capacity in promoting blood circulation and

dispersing blood stasis. In clinic, it has been used to treat cardiovascular (Li et al., 2014) and cerebrovascular diseases (Wang et al., 1993). The typical biologically active alkaloid of *Ligusticum wallichii* is tetramethylpyrazine (TMP) (Fig. 2C). It was isolated in 1957 (Guo et al., 1983). This natural compound can gain access to the brain through the BBB and is absorbed rapidly without accumulated toxic effect. Several studies have highlighted TMP's pharmacological capabilities for treatment of coronary heart disease (Mak et al., 2017), ischemic stroke (Zhang et al., 2018), AD (Yang et al., 2017), and cancer (Shen et al., 2018). It would appear that most of them have focused on the effective molecular targets of TMP in connection with inflammation, oxidant stress, platelet, and apoptosis (Yuan et al., 2016).

TMP is well known to play an important role in neuroprotective effect. It has been reported that TMP protected hippocampal neurons against apoptosis through JNK/MARK signal pathway in cerebral I/R animal models (Zhong et al., 2016). Moreover, in the ischemia stroke model *in vivo*, TMP exerted its therapeutic effect via PI3K pathway. Inhibition of chemokine (C-X-C motif) receptor 4 (CXCR4) with its antagonist AMD 310 was coupled with activation of PI3K/Akt, PKC, and ERK and stimulated neural progenitor/precursor cells migrating to the injured region. Inhibitors such as LY294002 or Myr- $\psi$ PKC blocked this change indicating the molecular mechanisms of TMP's function (Kong et al., 2016). TMP usually combined with *Salvia miltiorrhiza* is extensively used in China for treating coronary heart disease, cardiac angina and atherosclerosis. The combination significantly improved cardiac function in reduction of apoptotic rate and myocardial infarct size (Huang et al., 2016). Compounds including TMP created a better microenvironment against neuroinflammation and a lower rate of apoptosis. Application of the compound Danshen-Chuanxiong-Honghua increased neurogenesis and expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. Thus, the spatial cognitive deficit induced by MCAO was reversed (Zhang et al., 2017). Arising from the above, it seems evident that TMP has a neuroprotective function against apoptosis, especially in hippocampal neurons. In addition, TMP has been demonstrated to have beneficial effects on anti-inflammatory activity in ischemic stroke. Experimental studies pointed out that TMP enhanced expression of Nrf2/HO-1 and down-regulated high-mobility group box-1 protein (HMGB1)/toll-like receptor-4 (TLR4), Akt and ERK signal pathways. Recent studies have focused on the amelioration function of CXC195, a novel TMP derivative. CXC195 was found to regulate endothelial nitric oxide (eNOS) synthase phosphorylation (Yan et al., 2015) and anti-apoptosis (Chen et al., 2014a) after cerebral ischemia-reperfusion injury via PI3K/Akt/GSK3 $\beta$  signaling pathway. It increased NO production and Bcl-2 expression, and restored phosphorylation of eNOS and

Akt, leading to a significant reduction in infarct size and improvement in neurobehavioral outcomes. The role of TMP in neuroprotection and anti-apoptosis may be blocked by wortmannin, an inhibition of PI3K.

TMP impacted microglia mostly on analgesia and neuroinflammation. Chronic pain in periorbital followed by traumatic brain injury is readily alleviated by TMP. It attenuated microglia-induced neuroinflammation via the NF- $\kappa$ B signaling pathway (Wang, et al., 2017c). iTRAQ technology has confirmed the anti-inflammatory effects of TMP in suppressing microglia activation (Pu et al., 2015). It is to be noted that TMP can alleviate spinal cord injury by inhibiting activated microglia, thus repressing the expression of TNF- $\alpha$  and IL-1 $\beta$  (Shin et al., 2013). Neurodegenerative diseases, such as AD, PD etc. have been reported to be closely related to neuroinflammation induced by activated microglia which release excess amounts of proinflammatory and neurotoxic factors. Persistent microglia activation can lead to tissue injury. The neuroprotective potential of TMP lies in its efficacy by inhibiting proinflammatory cytokines and intracellular ROS release from primary microglial cells stimulated by A $\beta$ 25-35 and interferon- $\gamma$  (IFN- $\gamma$ ) (Kim et al., 2014b). TMP thus played an important role in modulating microglia activation as well as reducing the expression of proinflammatory cytokines and relevant mRNA (Shin et al., 2013). Many studies have reported the neuroprotective effect of TMP against ischemic stroke. Macrophages/ microglia activation and lymphocytes/neutrophils/macrophages infiltration are decreased by TMP thereby decreasing neuroinflammation. It has also been proposed that TMP can modulate macrophages/microglia activation by augmenting the expression of Nrf2/HO (Kao et al., 2013; Chang et al., 2015). It alleviated the damage caused by excessive production of NO and iNOS in LPS-induced N9 microglial cells, and the phosphorylation of p38 MAPK, ERK1/2, JNK and Akt simultaneously (Liu et al., 2010). It exerted both anti-apoptosis and anti-inflammatory effects in focal cerebral ischemic/reperfusion model. TMP also regulated Bcl-2/Bax/Bad expression, and caspase-8/caspase-9/caspase-3 activation (Kao et al., 2006).

### Effects of Scutellarin on microglial activation in cerebral ischemia

*Erigeron breviscapus*, a traditional herbal medicine native to Yunnan province, China, has been widely used for treatment of cerebrovascular disease (Cao et al., 2008; Pengyue et al., 2017). The bioactive compounds, including Scutellarin, 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid (Fig. 2D), have been isolated and explored in depth for their neuroprotective roles. Scutellarin (4,5,6-trihydroxyflavone-7-glucuronide), the dominating bioactive component of breviscapine, was reported to promote neurogenesis, protect neurons against damage, and possess antioxidant (Liu et al., 2005) and anti-apoptotic properties (Zhang et al., 2009;

Tang et al., 2014). Scutellarin increased cell viability, expression of mRNA, Protein Kinase G (PKG) and vasodilator-stimulated phosphoprotein (VASP) following cerebral ischemia reperfusion *in vivo* and *in vitro* (Du et al., 2015). Scutellarin, and its main metabolite (6-O-methyl-scutellarein) possesses protective effects against neuronal injury caused by ischemia/reperfusion (Wu et al., 2017b). In treatment of neurodegeneration diseases, Scutellarin could inhibit apoptosis of neural stem cells (NSCs) and promote differentiation of NSCs to myelin-producing oligodendrocytes, and then alleviate motor deficits for multiple sclerosis *in vivo* (Wang et al., 2016). 6-chlorotacrine-scutellarin hybrids can inhibit human acetylcholinesterase and human butyrylcholinesterase activation and prevent antioxidation (Spilovska et al., 2017). Surprisingly, Scutellarin treatment significantly prevented human hepatocellular carcinoma cell migration and invasion by downregulating the expression of STAT3/Girdin/Akt (Ke et al., 2017). Scutellarin protects apoptosis from prostate cancer by activating the caspase cascade suggesting its potential therapeutic value for prostate cancer patients (Gao et al., 2017).

Considering the broad spectrum and safety of Scutellarin in clinical trials, it is likely to be a valuable dietary supplement in the treatment of cerebral ischemia and neurodegenerative diseases. As discussed above, activated microglia execute dual but opposing roles in neurodegenerative diseases by clearing the cellular debris and invading pathogens and releasing neurotrophic factors etc.; on the other hand, they also cause harmful consequences by releasing excessive inflammatory mediators especially when they are over-activated. Therefore, suppressing microglial over-reaction and microglia-mediated neuroinflammation has been considered an appropriate therapeutic strategy for amelioration of neurodegenerative diseases. To this end, Scutellarin has been considered a potential candidate (Yuan et al., 2014). Scutellarin was confirmed to protect BV-2 microglial cells from LPS-induced neuroinflammation by reducing the expression of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, NO, and iNOS through AKT/NF- $\kappa$ B pathway. Meanwhile, it inhibited the phosphorylation of p38, JNK, and AKT via p38/JNK pathway (You et al., 2018). Remarkably, Scutellarin was reported to not only suppress microglia activation in middle cerebral artery occlusion in the infarcted areas, but also promoted astrogliosis coupled with improvement of neurological scores (Fang et al., 2016). Studies conducted in our laboratory revealed that Scutellarin was very effective in suppressing excessive release of proinflammation mediators IL- $\beta$ , IL-6, TNF- $\alpha$ , NO and ROS. Furthermore, it suppressed the expression of Notch-1 and its downstream members of the pathway as well as NF- $\kappa$ B, resulting in decreased release of proinflammatory mediators. It promoted the rearrangement or reorganization of microglia cytoskeleton and improved tissue remodeling after an ischemic insult in rats (Yuan et al., 2015). Remarkably,

conditioned medium derived from activated microglia pretreated with Scutellarin amplified the astrocytic reaction (TNC1 astrocytes) *in vitro* indicating a cross-talk between the glial cell types (Fang et al., 2015a; Wu et al., 2017a).

Recent studies on Scutellarin have focused on its effects on HIE. Scutellarin administration (50 mg/kg/d) for 30 days has significantly alleviated cognitive impairment after hypoxia. It facilitates proliferation of the NSCs and neuronal differentiation (Wang et al., 2017b). In addition, Scutellarin protects rat cortical neurons from OGD-induced toxicity by augmenting the cellular antioxidant defense capacity (Guo et al., 2011). Scutellarin also promotes the production of neurotrophic factors through p-CREB and p-Akt signaling to mitigate the toxicity induced by hypoxia (Chai et al., 2013). Other studies also evaluated the neuroprotective effect of Scutellarin showing that the expression of LDH and its mRNA was reduced in PC12 cell in hypoxia/reperfusion model (Xu et al., 2007a). Despite the above, the effects of Scutellarin on HIE remains to be fully explored. In our own study, Scutellarin at a high dosage of 20mg/ml was found to decrease the expression of TNF- $\alpha$ , and iNOS at 24 hours after the onset of HIE *in vivo*.

#### Effects of Gastrodin on microglia activation in cerebral ischemia

Gastrodin (p-hydroxymethylphenyl- $\beta$ -D-glucopyranoside) (Fig. 2E), the main bioactive compound isolated from *Gastrodia elata* Blume (*G. elata*), is a traditional herb that has been widely used in treatment of disease in the CNS (Song et al., 2013). Gastrodin has been used for treatment of vertigo (Xie et al., 2014), convulsive illness (Wong et al., 2016), headache (Choi et al., 2011) and sedation (Lin et al., 2008). There is strong evidence supporting that Gastrodin plays an important role in neuroprotective effects on neurons. In the rat model of PD, Gastrodin can prevent diminution of dopaminergic neurons from toxin-induced apoptosis (Kumar et al., 2013), inhibit IL-1 $\beta$  expression and neuroinflammation induced by rotenone in the substantia nigra (Li et al., 2012), and promote the expression of HO-1 through p38 MAPK/Nrf2 signaling pathway. Gastrodin promotes primary neural progenitor cell viability from amyloid  $\beta$  (A $\beta$ ) (1-42)-induced neurotoxicity, improves hippocampal neurogenesis, decreases the incidence of apoptotic cells and alters expression of apoptosis-related proteins (Li and Qian, 2016). Other studies also reported that Gastrodin alleviates memory deficits (Zhang et al., 2016) as well as decreases the lesion of amyloid- $\beta$  peptide deposition in the cerebral cortex and hippocampus of rats (Zhao et al., 2012; Chen et al., 2014b). All these results have provided strong evidence of a potential merit of Gastrodin in clinical usage for PD or AD (Hu et al., 2014).

There is only a modicum of information on the effects of Gastrodin in treatment of HIE. It has been

reported that Gastrodin downregulated the expression of NR1 mRNA of N-methyl-D-aspartate (NMDA) receptor (Fu et al., 2008), abolished hypoxia-, glutamate- and NMDA receptor-induced toxicity in primary culture of rat cortical neurons, and protected cultured hippocampal slices against NMDA toxicity (Wong et al., 2016). Moreover, with pretreatment with Gastrodin (100 ug/ml) in hypoxia, neuronal viability was remarkably enhanced after 24 hours of hypoxia. Simultaneously, glutamate- and NMDA-induced neurotoxicity was decreased (Xu et al., 2007b). Gastrodin pretreatment with the dosage of 15 ug/ml and 30 ug/ml decreased excessive  $Ca^{2+}$  and NO as well as cell death induced by OGD (Zeng et al., 2006).

Microglia in the CNS are characterized by the heterogeneity of phenotypes. When stimulated by either external or internal stimuli, they are readily activated and polarized into M1 or M2 phenotype such as in cerebral ischemic injury. It would appear that the balance between M1 and M2 microglia/macrophage population may affect the outcomes of the cerebral damage. The beneficial effects of Gastrodin are evident in murine RAW264.7 macrophages as it promotes upregulation of the mRNA expression of Mgl2 and Mrc1 and arginase activity. Separately, Gastrodin was found to stimulate polarization toward M2-like macrophages with improved motor performance in cerebral palsy patients (Jia et al., 2017). Though there is still lack of sufficient evidence of its beneficial effect in HIE, Gastrodin remains an effective traditional herbal for treatment of neurological diseases in which neuroinflammation is implicated including HIE.

Recent experimental evidence has demonstrated that Gastrodins acts as a potent therapeutic agent in suppressing activated microglia. In a separate study conducted in our laboratory, it has been shown that Gastrodin ameliorated neuroinflammation in modulating renin angiotensin system (RAS) in activated microglia in hypoxic-ischemia neonatal rats as well as in LPS stimulated BV-2 microglia [unpublished observations]. Thus, Gastrodin was found to exert a neuroprotective function as it decreases the expression of nicotinamide adenine dinucleotide phosphate oxidase-2 (NOX-2), while increasing the expression of Sirtuin 3 (Sirt3) in hypoxic-ischemia induced microglia activation (Li et al., 2018).

Extracellular inflammatory peroxiredoxin (Prx) signaling was considered to be one of the crucial links for neuroinflammation after acute ischemic stroke. Gas-D, a derivative from Gastrodin, has been reported to diminish inflammatory responses stimulated by Prx1-, Prx2-, and Prx4 *in vitro*. It also protects neurons, microglia, macrophages, T-lymphocytes from ischemia damage after MCAO in rats (Mao et al., 2017). Additionally, Gastrodin reduced the activation of astrocytes and microglia with a concomitant decreased expression of TNF- $\alpha$  and IL-6 in the anterior cingulate cortex (ACC) of the CFA-injected mice. It was reported to relieve chronic pain, exert anxiolytic effects, and mitigate the inflammatory response induced by Freund's adjuvant (CFA) (Sun et al., 2016b). In BV-2 microglia,

Gastrodin significantly inhibits the NF- $\kappa$ B signaling pathway and phosphorylation of MAPKs. Thus it effectively modulates the expression of neurotoxic proinflammatory mediators in LPS-stimulated microglial cells (Dai et al., 2011). In addition, intraperitoneal injection with Gastrodin was shown to remarkably bring down pro-inflammatory mediators by activated microglia in models of acute ocular hypertension (AOH) (Wang et al., 2017a). All these suggest that Gastrodin has a potential protective effect in hypoxic-ischemia damage in which it can effectively dampen neuroinflammation mediated by microglia activation.

In comparison with some common drugs/agents that are endowed with anti-inflammatory and antioxidant properties, such as minocycline, melatonin, resveratrol etc., the five natural compounds discussed in this review present fewer side-effects, if at all, in clinics. Though minocycline was shown to effectively inhibit neuroinflammation mediated by activated microglia and modulate the polarization of microglia (Ahmed et al., 2017), its usage is limited such as for the treatment of *Neisseria meningitidis* in view of its vestibulotoxicity. Recent studies have also highlighted the role of melatonin in suppressing the activated microglia through TLR4/ NF- $\kappa$ B signaling pathway or TLR4 dependent caspase-3 signaling after hypoxic-ischemic brain damage (Yao et al., 2016; Hu et al., 2017). Although melatonin has beneficial effects in neuroinflammation along with regulation of the immune-pineal axis, and prevention of BBB damage in cerebral ischemia (Jang et al., 2012), intracerebral hemorrhage (Wang et al., 2018), and even in spinal cord injury (Piao et al., 2014), there is no available evidence as in the herbal agents whether it can also secrete neurotrophic factors to exert a neuroprotective role specifically in cerebral ischemia. Resveratrol which has been widely studied in recent years has been found to gain access readily in the brain tissue via the BBB. It was used to decrease the expression of proinflammatory mediators from astrocytes and microglia in AD (Zhao et al., 2018). It was reported to possess many beneficial effects including anti-oxidative, anti-inflammatory and modulation of immune in different neurodegenerative diseases (Pannu and Bhatnagar, 2019). In our recent study, we have found that scutellarin in combination with edaravone was capable of reducing cerebral infarct size and improving the neurological score (unpublished data). On account of its rapid metabolism, low bioavailability, and complexity of its structural binding properties (Saqib et al., 2018), the effective use of resveratrol clearly needs to be further investigated for better comparison with the herbal agents here discussed.

## Conclusions and opinions

Cerebral ischemia remains a debilitating disease with neurologic impairment and high mortality rate in our ageing population. The ongoing search to identify potential drug candidates including active ingredients or compounds from natural products will continue to be the

focus of many studies. It is apparent from the present review that management of microglia-mediated neuroinflammation is crucial to effectively reduce brain damage.

While it is unequivocal that all five natural products mentioned here have anti-inflammatory and anti-oxidant properties especially in cerebral ischemia (Fig. 1), it remains to be explored how they would act on other glial types in the brain. Already there is convincing evidence that they can act specifically on activated microglia, a key player in neuroinflammation; yet their effects on other cellular components in the CNS remain uncertain. For example, their effects on astrocytes, oligodendrocytes as well as synaptic structures have not been fully investigated. It is also uncertain if they act singly or collectively on various signaling pathways that govern the production of proinflammatory mediators in activated microglia. Apart from gastrodin, the herbal agents here discussed can exert effects on angiogenesis. In this regard, they have been found to inhibit angiogenesis in many types of tumor. On the other hand, the impact of some herbal agents such as ginsenoside in promoting angiogenesis should be considered to slow down the tumor growth (Wu et al., 2017c). Both ginsenoside and scutellarin can promote retina repair through HIF-1 $\alpha$  pathway from hypoxia/ischemia brain injury (Wang et al., 2014a). Additionally, all five natural products can help prevent BBB damage and improve brain remodeling in modulating stem cell differentiation following cerebral ischemia. All in all, it is safe to state that all five common herbal agents as discussed here are endowed with anti-inflammatory, anti-oxidant and anti-apoptotic properties that can modulate microglia activation as evident in different experimental paradigms. Future work should be focused to harness these properties for designing effective strategies for treatment of cerebral ischemia or other neurodegenerative diseases. One possibility, for example, would be to consider using the herbal compounds separately or in combination in balancing the polarization of M1 and M2 microglia towards the protective phenotype. Identification of specific receptors and signaling pathways in microglial activation via which these compounds might act either directly or indirectly would be of paramount importance. Finally, the possibility that they might regulate the microglial activation epigenetically such as through histone modifications and microRNAs (Patnala et al., 2017) should be the future scope of study.

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