



Pathophysiology of ischemic reperfusion injury and the molecular targets involved in amelioration of brain injury by herbal medicine

Ibrahim Mohammed Badamasi^{1,A-F} 

¹ Bayero University, Kano, Nigeria

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Abstract

Introduction and objectives. A number of preclinical evaluations of stroke treatment with herbal medicine (HM) have been reported. The aim of the current review was to highlight the pathophysiology of stroke and review the pre-clinically identified molecular mechanisms of HM treatment.

Materials and method. Only 32 articles published in the English language were accessible on Google scholar describing the treatment and mechanistic processes of HM in animal models of stroke, as well as human clinical trials, and were reviewed in this study.

Results and discussion. Suboptimal Na⁺/K⁺ ATPases pump activity, actions of microglia cytokines that increase the level intracellular adhesion molecules-1 (ICAM-1) which promote WBC extravasation with associated increased in matrix metalloproteinase (MMP) activity (digest basement-membranes), explains edema and apoptosis/inflammation. Altered conductivity in injured neurons with compensatory increase in glutamate release that overwhelms the regulatory glial glutamate transporter 1, and thus peaks the level of glutamate to an excitotoxin level, promotes neuronal death. Glutamate activity on NMDAR promotes oxidative stress, lipid peroxidation and release/influx of Ca²⁺ that causes apoptosis. The molecular targets involved in the treatment for stroke by HM promote anti-apoptotic/anti-inflammation, anti-oxidation, angiogenesis, neurogenesis, anticoagulation/fibrinolysis effects and optimal metabolism. Different HM promotes the activities of tissue plasminogen activator, haemeoxygenase 1, Neutrin-1, brain derived neurotropic factor (BDNF) and **mitogen-activated protein kinase** (MAPK).

Conclusion. The pathophysiology of stroke and the preclinical targets on which HM act to ameliorate them were identified which could serve as a focus for research on the development of effective treatment for stroke.

Key words

brain, molecular, efficacy, stroke, complimentary medicine

INTRODUCTION

Brain stroke is commonly characterized by a region of cell death attributed to ischemic changes, as well as an active adjacent area of brain tissue regeneration called a penumbra [1, 2]. A high level of vasculature as well as early re-establishment of functional microvasculature around the stroke region facilitate quick and optimal recovery of neuronal cells after stroke [3]. The reperfusion of the ischemic region, which is apparently necessary to salvage the neuronal cells from irreversible damage, has also been implicated as a major source of injury to the brain [4]. Increased reactive oxygen species, dysregulation of calcium hemostasis, microvascular dysfunction with loss of blood flow, exaggeration of inflammatory response, and slow-onset apoptotic cell death contributes to reperfusion injury after ischemia [5]. There are numerous possible causes of ischaemia reperfusion injury, such as trauma, hypovolemic shock with resuscitation, vascular reflow after contraction, percutaneous transluminal coronary angioplasty, thrombolysis treatment and organ transplantation [6].

Most conventional therapeutic agents used for the management of ischemic stroke have single mechanisms of action geared towards revamping, as well as maintaining the blood flow, in order to optimize the functioning of neurons and not necessarily the functions of other brain supportive structures [7, 8, 9]. Tissue plasminogen activator (tPA) as well as mechanical thrombectomy are the only treatment approved by the FDA in the USA for the management of acute ischemic stroke to reestablish blood flow [10]. The narrow therapeutic window for tPA, the short time period (3–4.5 hours) within which its administration potentially promotes neuronal recover, as well as the serious side-effects in many patients following its use, have limited its use among of patients eligible for treatment with tPA [11, 12, 13, 14]. Medications with multi-target capabilities for ameliorating/reversing individual pathological components of an entire framework of a complex pathology such as stroke, are potentially better fitted for achieving a remarkable outcome compared to medications with single-target remedy approaches to such pathology [15].

Herbal medicines (HM) are known to have multiple targets through which they promote the hemostasis of the entire neurovascular units comprising of neurons, endothelial cells, astrocytes and basal lamina [12, 16]. Therefore, the HM have great potentials for attenuating all the facets of distortions

Address for correspondence: Ibrahim Mohammed Badamasi, Bayero University, Kano, Nigeria

E-mail: badamasi_mohammedibrahim@yahoo.com

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that are vital for the development of detrimental effects of stroke, and hence they are potentially very promising candidates for supporting standard stroke treatment. A double-blind, placebo-controlled and randomized clinical trial identified that the use of *Gingko biloba* at 120mg/day for four months during the post-stroke period, not only hastened recovery but also decreased the scores according to the National Institute of Health Stroke Scale (NIHSS) [17]. A meta-analysis and double-blind, placebo-controlled and randomized clin, with associated optimal functional recovery [18, 19].

OBJECTIVES

A detailed update of the pathophysiological processes for the development of stroke, as well as a systematic review of the pre-clinically identified mechanisms involved in the activity of HM for stroke management, were the main focus of the study. Highlighting the molecular mechanisms of actions of these HM could serve as a nidus for identifying clinically-druggable targets for new medicines that are both safe and effective for the management of stroke. Figure 1 shows the general pathophysiological features associated with cerebral ischemic hypoxic injury to the brain, as well as the mechanism of activities of HM that may resolve/attenuate the different segments of the pathology.

MATERIALS AND METHOD

The processes adopted for the current review involved a thorough search of the Google scholar data base using search

such word as ‘neurogenesis’ and ‘herbs’. The criteria included that the articles must be of either human, animal or *in vivo* studies, and must be related to the role of an HM in stroke and/or neurogenesis. The scope of the articles may include evaluation of the modulatory effect of HM on stroke patients and /or stroke model, and therefore be related to preclinical or clinical studies. A total of 6,040 articles identified were by using the search words were evaluated. The search out-put was arranged as 10 article titles per page for a total of 100 pages, and all articles were evaluated. The titles relevant to the proposed review were further assessed by reading the abstracts and the whole articles (32) and thus identified as fulfilling the criteria for inclusion in the current review (Fig. 2).

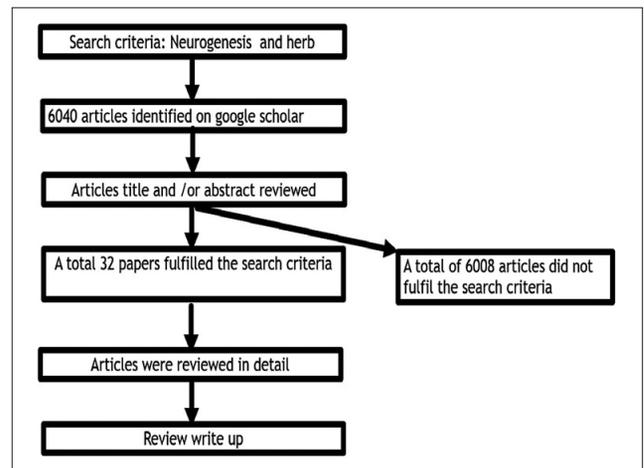


Figure 2. Step-by-step methodology involved in the identification and selection of reviewed articles in the current review

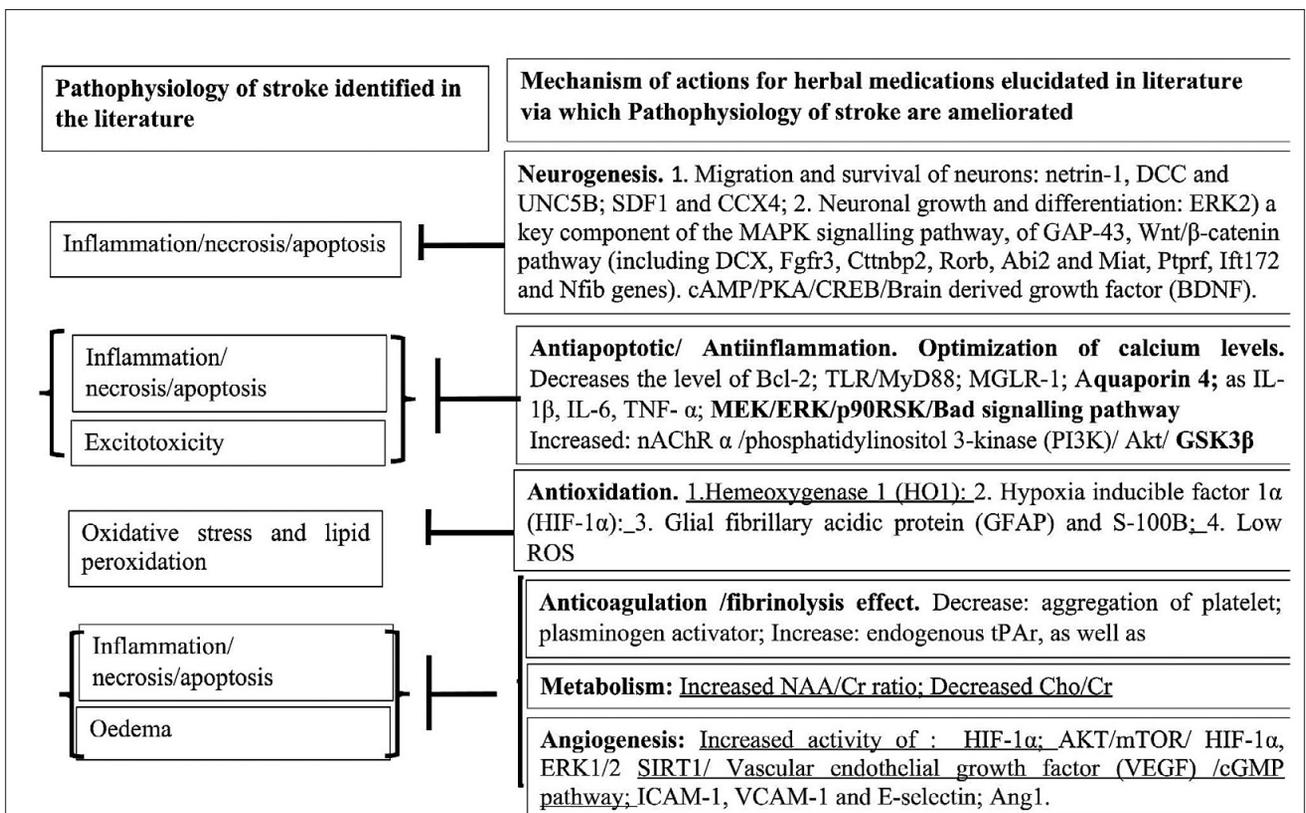


Figure 1. Overview of the pathophysiology of stroke and molecular targets on which herbs have been elucidated to act

DESCRIPTION OF THE STATE OF KNOWLEDGE

Pathophysiology of stroke. The events that culminate in the pathology of stroke are derived from both the initial insult of cerebral ischemia-hypoxia and those of the reactive responses following cerebral reperfusion that are aimed at repairing the damaged tissue (Fig. 3, 4). Cerebral ischemia/reperfusion leads to increased levels of free radical, excitatory glutamate, and intracellular calcium overload, as well as inflammation and its products, which release active matrix metalloproteinase (MMP), as well as dissolution of the basement membrane which disrupts the integrity of the **blood brain barrier** (BBB) with the risk for cerebral edema and hemorrhage [20, 21, 22]. The BBB is a barrier against the admixture of extracellular fluid of CNS with the blood in the blood vessels of the brain [23]. Tight junctions between endothelial cells are vital constituents of the BBB. Claudins, occludin, junctional adhesion molecule, as well as other cytoskeletal proteins, are key constituents of tight junctions and can potentially be affected by physiological and pathological conditions [24, 25, 26, 27]. Loss of BBB, Cerebral edema and hemorrhagic transformation of an ischemic stroke, are a nidus for the exacerbation of brain injury [27]. Swelling of the brain caused by edema can increase intracranial pressure, with a sustained ischemic injury and formation of a hernia [28].

KEY PATHOPHYSIOLOGICAL EVENTS OF STROKE

Inflammation. One hour after cerebral ischemia, a progressive increase in the permeability of the brain blood vessels begins and usually last for a day. This disruption of the BBB permits proteins as well as fluid to enter the cerebral extracellular space with associated vasogenic edema and brain injury (Fig. 3) [28, 29]. Sterile inflammation is triggered by the cerebral ischemic injury that activates brain astrocytes and microglia cells (key regulators of brain endothelial cell)

and promote the formation of inflammatory cytokines **such as interleukin one-beta (IL-1 β), interleukin -six (IL-6), and tissue necrosis alpha (TNF- α)** that exacerbate the inflammation further [23, 30, 31, 32]. TNF- α promote the expression of **intracellular adhesion molecules -1 (ICAM-1)**, which accelerates the adhesion and extravasation of leukocyte into the region of brain injury [23]. The brain injury also promotes the infiltration of macrophages and leukocytes which also promote the disruption of BBB [23]. The leucocyte dependent change from transient to persistent disruption of the BBB-tight junction protein orchestrated by neutrophil-derived neurovascular metalloproteinase-9 (MMP-9), is key in the worsening of Ischemic brain injury[6]. It is pertinent to note that elevated levels of IL-1 β , IL-6, and TNF- α play a central role in post-stroke inflammation induced neuronal death (Fig. 3) [12]. Apoptotic cell death may also be elicited by the activities of these pro-inflammatory cytokines which can induce NF- κ B nuclear translocation with elevation of P53-upregulated modulator of apoptosis (PUMA) (Fig. 3). When p53 is released from **B-cell lymphoma-extra large (Bcl-X_L)** by activated PUMA, it can activate Bax and results in apoptosis within the infarcted hemisphere (Fig. 3, 4) [33].

Excitotoxicity. Excessive glutamate within the brain extracellular space is common following cerebral hypoxia-ischemic injury and it serves as an excitotoxin which promotes slow onset of neuronal death [34, 35]. Excess glutamate is usually removed by glial glutamate transporter1 (GLT-1), and is therefore responsible for maintaining the glutamate levels below neurotoxic levels (Fig. 4) [36]. In preclinical studies, the regions of the brain that were observed to have increased expression of GLT-1 after hypoxia-ischemic injury (such as **Cornu Ammonis-three (CA3)** of the hippocampus, show lower levels of apoptotic neuronal death when compared to those regions without an increase in GLT-1 (such as **Cornu Ammonis - one and two (CA1 and CA2)** of the hippocampus)

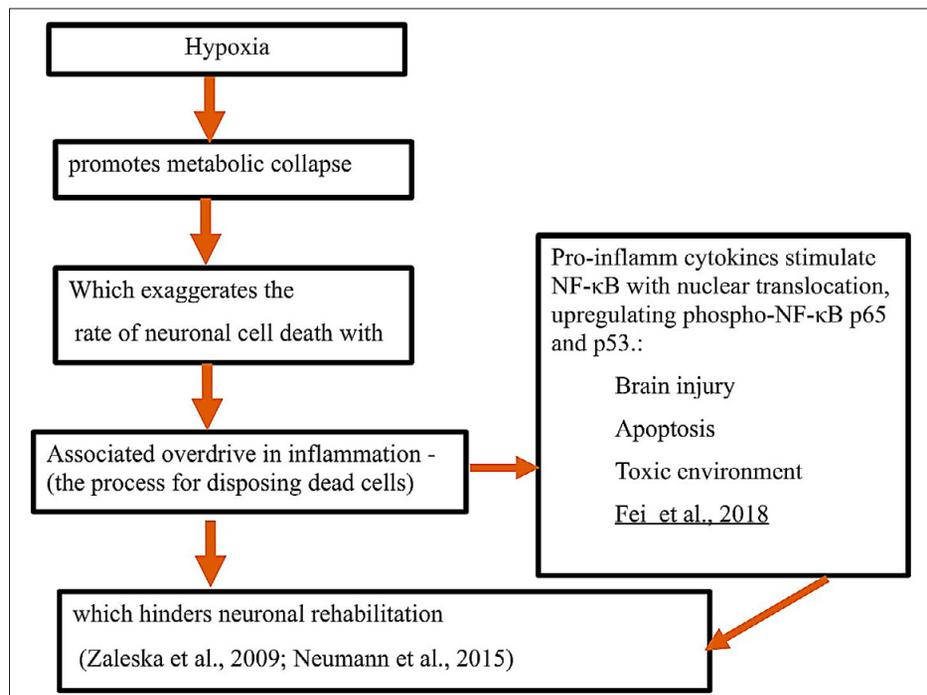


Figure 3. Pathophysiology of stroke in the current review

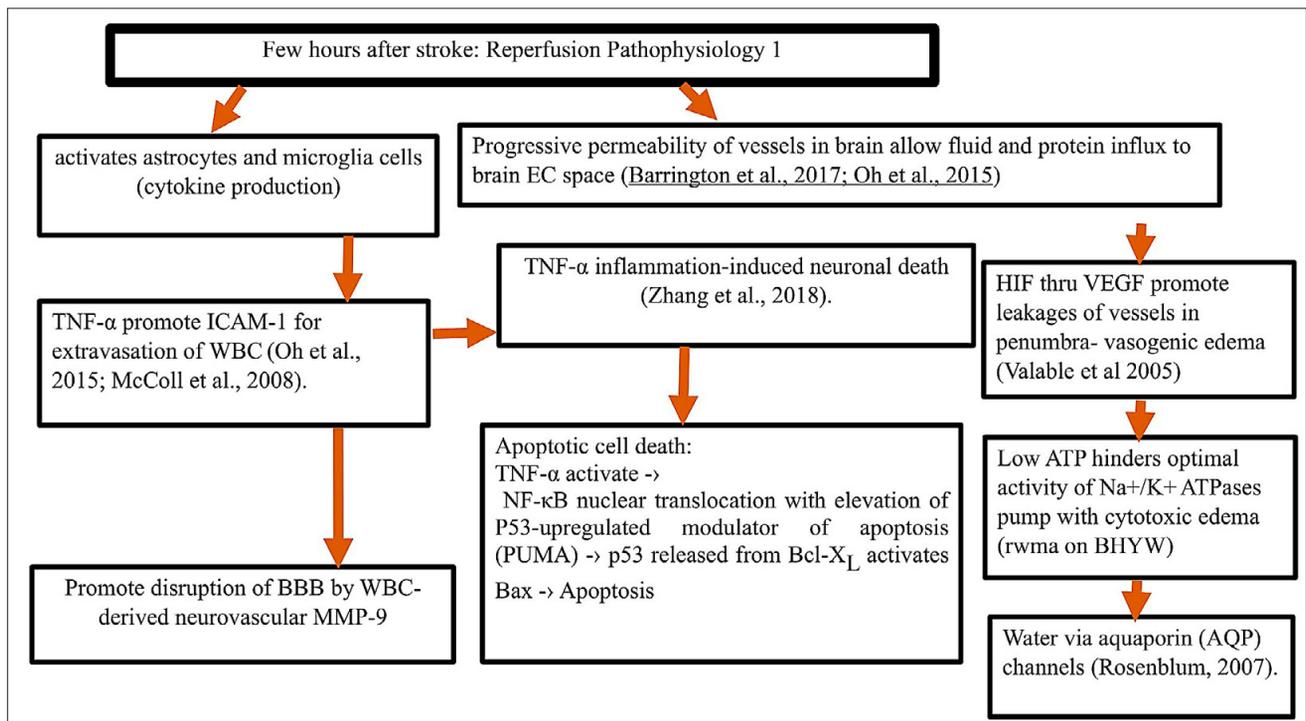


Figure 4. Events that occur few hours after stroke. Reperfusion pathophysiology 1

[37]. Excessive levels of potassium and calcium have also been reported to be promoters of poor neuronal excitability as well as an increase in the death of neurons via apoptosis [5, 38]. The degradation of the intracellular homeostasis for calcium involves a number of cellular factors, such as depletion of ATP following ischemic – hypoxic insult to cerebral tissue. The depleted ATP renders **sodium ion – potassium-ion-Adenosine triphosphatase** (Na^+, K^+ -ATPase) enzyme which regulates membrane ionic concentration highly incapacitated with associated persistent depolarization, as well as the opening of voltage-gated calcium channels (VGCCs) [39]. The ischemic-mediated increase in the release of glutamate (excitatory neurotransmitters) associated with activation of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, further depolarizes the membrane. It also increases the activation of N-methyl-D-aspartate receptor (NMDAR) which promotes **nicotinamide adenine dinucleotide phosphatase** (NADPH) oxidase or mitochondria-dependent **reactive oxygen species** (ROS) generation, with release of stored intracellular Ca^{2+} as well as influx of more Ca^{2+} via transient receptor potential melastatin (TRPM) channels (Fig. 4) [40, 41]. Calcium permeable acid-sensing ion channel 1a (ASIC1a) has also been identified that may allow the influx of calcium following acidulation changes associated with cerebral ischemic hypoxic injury (Fig. 5) [42].

Oxidative stress and lipid peroxidation. The high risk of oxidative stress for the brain tissue is due to its high rate of oxidative metabolic activity with associated high rate of ROS production. Its high content of polyunsaturated fatty acid, low levels of antioxidants and poor resilience to damage, repair and replication of neuron, also contribute to its high risk of oxidative stress (Fig. 6) [43]. Generally, oxidative stress and lipid peroxidation are closely associated with ROS (Superoxide and hydroxyl radicals) which act as a potent

destroyer of membrane via lipid peroxidation. High levels of lipid peroxides are reported in brain and muscle following ischemia-reperfusion injuries [44, 45].

Edema. A common response to ischemia or hypoxia in the brain involves the production of a hypoxia inducible factor [46]. Downstream genes encoding erythropoietin, vascular endothelial growth factor (VEGF) and glucose transporters, usually mediate the activity of HIF [47]. VEGF is rapidly expressed in the penumbra of brain ischemia region and promotes leakages of vessels as well as activation of MMP-9. The overall activity of hypoxia inducible factor 1 α (HIF), as well as its downstream proteins, are related to the development of a vasogenic edema in the early stages after ischemic stroke (Fig. 4).

Direct transportation of water into cells via aquaporin (AQP) channels can also lead to cell edema in the presence of an intact BBB [48]. The epithelial sodium channel (ENaC) has also been reported to play a central role in the regulation of fluid balance in the cerebral tissue [49, 50]. Failure of the Na^+ / K^+ ATPase pump after the loss of adenosine triphosphatase (ATP) associated with ischemia/hypoxia, may underlie the development of cytotoxic edema mediated through the ENaC (Fig. 4) [44].

Molecular targets involved in the herbal medicine treatment for stroke. Figure 1 shows the overview of the various mechanism of action of herbal medicine that for many years have played a central role in the management of stroke. The key mechanisms essentially include either an elevation or decrease in important direct or indirect mediators for cerebral ischemic injury. The key mechanism highlighted in Figure 1 include: antioxidation; neurogenesis; anticoagulation /fibrinolysis; angiogenesis and metabolism related processes.

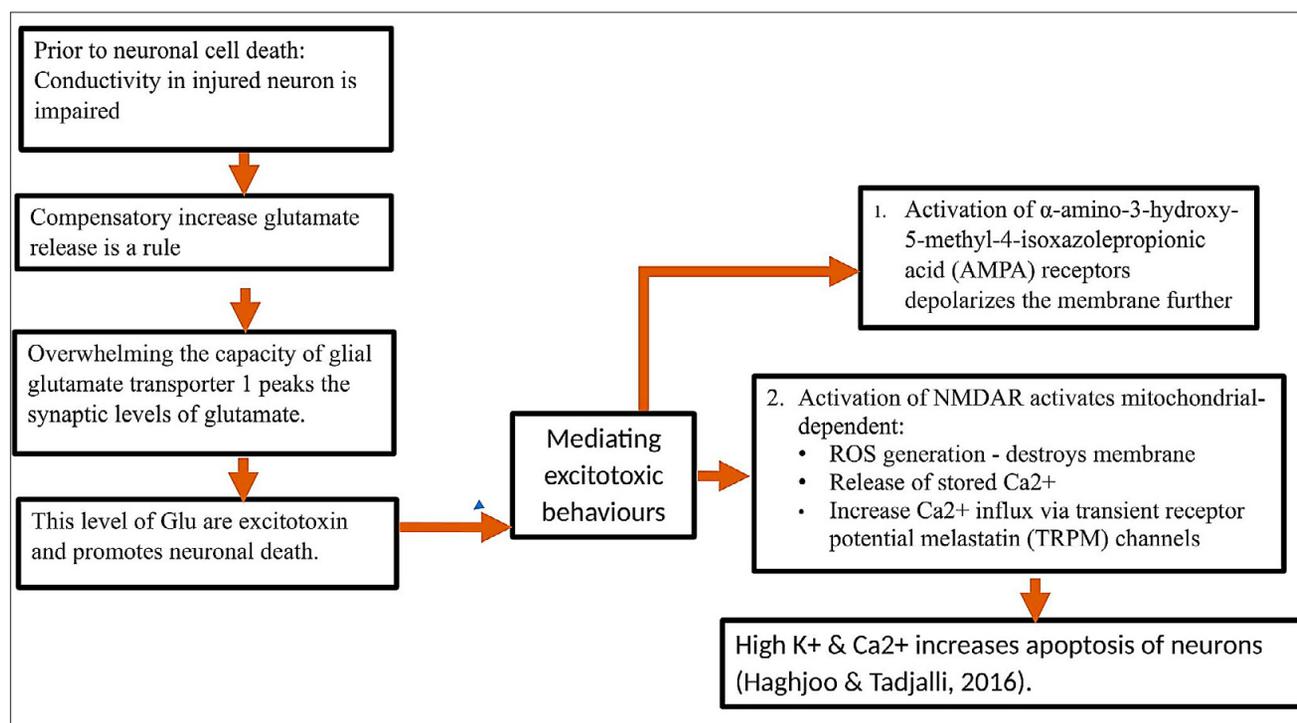


Figure 5. Events that occur prior to neuronal death. Reperfusion pathophysiology 2

Antioxidation – Hypoxia inducible factor 1 α . A number of antioxidation-related features have been identified in the mechanisms of action for herbal medicines that promote resolution of cerebral ischemic injury in the literature. Hypoxia inducible factor 1 α (HIF-1 α): the activity of hypoxia inducible factor – 1 α (HIF-1 α) is mediated mainly through the activities of downstream genes of erythropoietin, vascular endothelial growth factor (VEGF), CCXR4, SDF-1 and glucose transporter [47, 51]. Its activity, as well as the activities of the genes that it enhances (especially those of VEGF, and **chemokine receptor type 4 (CCXR4/ Stromal Cell Derived Factor 1 (SDF-1))** promote angiogenesis [51]. There are studies that implicate the activity of HIF-1/VEGF as well as ENac in the development of edema following cerebral ischemic stroke through vascular leaks and MMP-9 activation, which can potentially worsen the cerebral injury [50, 52]. It has also been directly attributed with a damaging effect on basement membrane, thereby promoting sterile inflammation as well as leakage of the BBB with an increased risk for edema (Fig. 5) [53, 54, 55]. The conundrum regarding the activity of HIF-1 α as to whether it is beneficial or deleterious, has been evaluated and the findings suggest that its detrimental inflammatory role after cerebral ischemia is associated with its early post-stroke peaking (hours after stroke, while its peaking after two to six days following stroke is beneficial [56]. Therefore, the HM such as *Buyang Huanwu Decoction (BHD)*; *Huatuo Zaizao pill (HZP)* that can optimize the stimulatory activity of HIF-1 α on the downstream genes involved in the angiogenesis pathway, are of immense potential benefit in the treatment of stro, especially when prescribed after the day of insult (Fig. 4).

Antioxidation – Hemeoxygenase 1 (HO1). This is an antioxidant enzyme primarily involved in the breakdown of heme. Its activity is optimal under stressful conditions, including hypoxic / ischemic injuries to the brain, which indicates a potential role for the enzyme in ameliorating

stroke related neuronal pathology [57, 58, 59]. There are studies reporting an increased expression of vascular endothelial growth factor (VEGF) and a collapse in response mediator protein 2 (CRMP2) (vital for neuronal development), mediated by an elevated level of HO1 antioxidant activity [60, 61]. Therefore, HO1 antioxidant activity promotes angiogenesis via VEGF as well as neuronal development and outgrowth via CRMP, which is a mechanism via which some HM **especially EGb 761 of G. biloba** promote neuronal wellbeing (Fig. 6) [62, 63].

Antioxidation – Decreased production of ROS. Attenuation of the production of superoxide anion (O₂⁻), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) by HM also ameliorates the degree of neuronal damage reported following brain I/R injury. This protective effect against ROS and its activity is completely lost following attenuation of the Toll-like receptor/ **Myeloid differentiation primary response 88 (My88)** pathway (Fig. 8) [64].

Neurogenesis. Proliferation, migration and differentiation of neuronal stem cells (NSC) are the key activities that characterize neurogenesis (Fig. 7) [65]. Neurogenesis, as well as axonal remodeling, usually occur in the subventricular zone (SVZ) of the lateral ventricle following stroke or trauma; however, the normal endogenous neurogenesis is usually insufficient to ensure adequate functional recovery [66, 67]. Thus, HM such as **Danshen-Chuanxiong-Honghua (DCH)**, **Ruyi Zhenbao (RZ)**, **Xijiao Dihuang Decoction (XDD)** and **extract of Ginkgo biloba 761 (EGb 761)** that promote neurogenesis can play a vital role in accelerating the neurogenesis process to a correspondingly optimal level for recovery from I/R injury (Fig. 7).

Migration and survival of neurons. Increased expression of netrin-1 and its associated receptors (DCC and UNC5B)

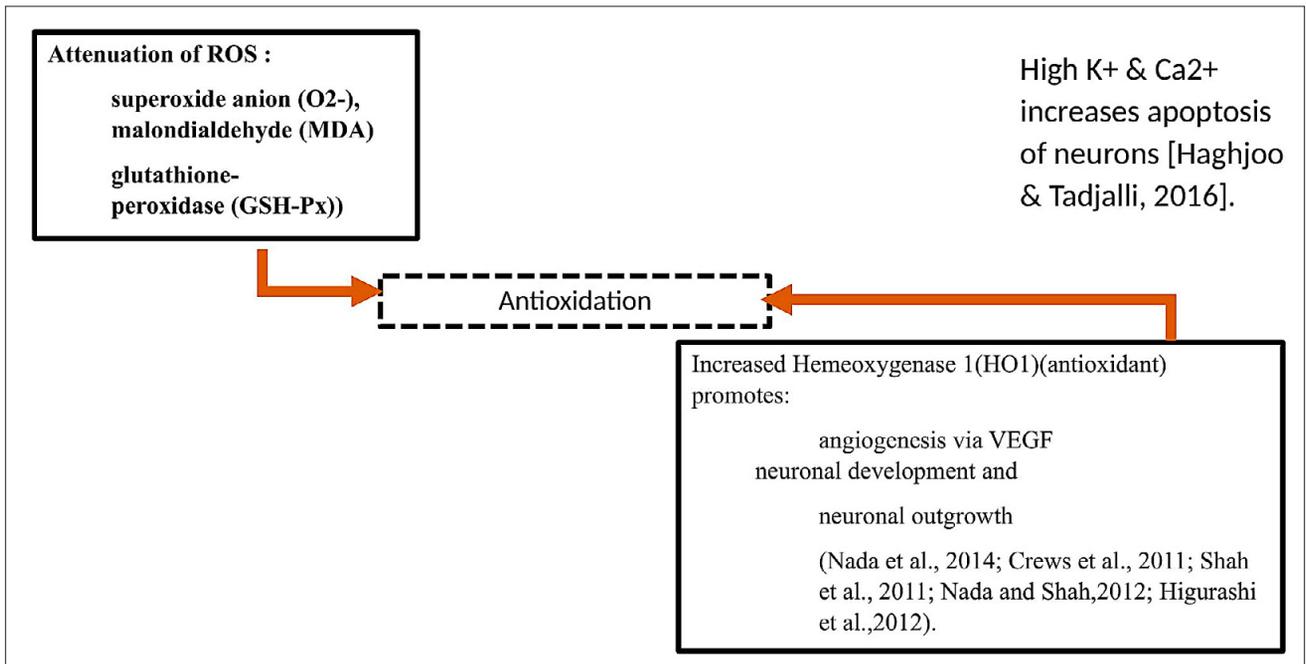


Figure 6. Molecular targets involved in the herbal medicine treatment for stroke (MTHM) via anti oxidation

promotes the activities of axons as well as the increased expression of **stromal cell derived factor1(SDF1)** and **chemokine receptor type 4 (CCX4)** which have also been associated with more stem cells in the penumbra [68, 69]. Therefore, these targets mediate taxis of neural stem cells (NSC) to the region of the penumbra and **HM**, such as **EGB 761**, that upregulate them will putatively promote NSC migration into the penumbra, as well as ameliorate the neuronal loss as site of I/R injury (Fig. 7).

Neuronal growth and differentiation. Increased expression as well as activity of extracellular signal regulated kinase 2 (ERK2, a key component of the **mitogen-activated protein kinase (MAPK)** signalling pathway which promote neuronal growth and differentiation in the ischemic area, has been reported [70, 71, 72, 73, 74, 75]. Another mechanism of action for herbal therapy associated with improvement of neuronal integrity following stroke, involves a post-stroke increased expression of **growth associated protein 43 (GAP-**

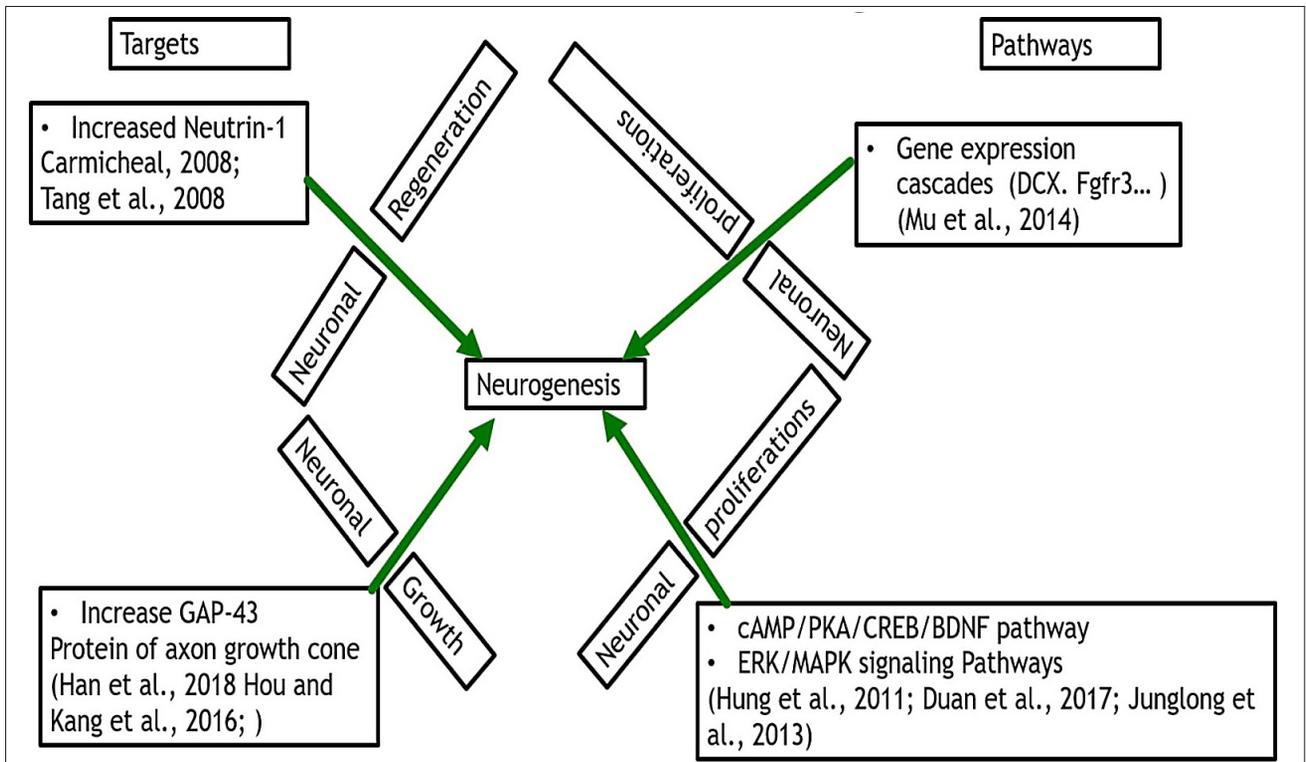


Figure 7. Molecular targets involved in the herbal medicine treatment for stroke (MTHM) via promoting neurogenesis

43), a phosphoprotein that is common at the growth cone of axons. It is commonly associated with neuronal rewiring following brain or spinal cord injury, and therefore its increased level following treatment with HM, such as ***Buyang Huanwu decoction (BHD)***, is an indicator of therapeutically-mediated axonal regrowth and remodelling (Fig. 7) [76, 77, 78].

Wnt/ β -catenin pathway for neural cell development. This pathway, when activated as characterized by increased expression as well as levels of **wingless-related integration site** (Wnt) ligand, promotes neurogenesis as well as the differentiation and survival of the newly formed neurons (Fig. 7) [79, 80]. It can be activated by one of the HM called Huatuo Zaizao pill (HZZP).

Genes for neural regeneration and development. The increased expression of DCX, Fgfr3, Ctnbp2, Rorb, Abi2 and Miat, as well as Ptpfrf, Ifi172 and Nfib genes by the activities of HM such as Houshiheisan (HSHS), have been associated with increased neuronal regeneration and development in preclinical evaluations (Fig. 7) [81].

cAMP/PKA/CREB/Brain derived growth factor (BDNF) for neural cell development. When **Brain derived growth factor** (BDNF) is elevated in the neural stem cells (NSCs), they characteristically promote neuronal proliferation and differentiation [82]. The activation of BDNF gene usually follows a biological cascade of events triggered by cerebral ischemia and promoted by therapeutically-mediated increased phosphorylation of **cAMP response element-binding protein** (CREB) by **protein kinase A** (PKA), thus generating phosphorylated-CREB (p-CREB). Phosphorylated-CREB can increase transcription of the BDNF gene by binding to its **cAMP response element** (CRE) element of the gene promoter region (Fig. 7) [82, 83, 84, 85]. HM such as HZZP and BHD are very potent activators of the cAMP/PKA/CREB/Brain derived growth factor (BDNF) pathway.

Glial fibrillary acidic protein (GFAP) and S-100B protein for neural cell development. Attenuation of the expression of glial fibrillary acidic protein (GFAP) and **S100 Calcium Binding Protein B** (S-100B), which probably arises as a consequence of both an antioxidation as well as an anticoagulation related effects of herbal medication, has also been reported [72, 86, 87]. Attenuation in the level of GFAP had been associated with uninjured healthy brain tissue [81], and is therefore a biological marker for healthy neuronal tissue (Fig. 8).

Antiapoptotic / Antiinflammation. Hypoxia following stroke promotes metabolic collapse which facilitates an exaggerated rate of cell death among neurons. The normal inflammation process is chiefly concerned with the disposal of both dead cells as well as necrotic debris, while overzealous inflammation after stroke inhibits neuronal rehabilitation [88, 89]. A number of molecular features have been highlighted in the literature that promotes antiapoptosis / anti-inflammation following cerebral ischemic injury, and include: optimization of calcium levels, attenuation of Toll-like receptor (TLR), optimization of glutamate level, increased activity of **nicotinic acetylcholine receptor α 7** (nAChR α 7) receptors, and attenuation of the activities of proinflammatory cytokines (Fig. 8).

Optimization of calcium levels. Optimization of calcium levels promote increase in the length of axons and the expression of neurofilament and GFAP [90, 91, 92]. Therapeutic strategies have also prevented intracellular calcium overload. Calcium overload is a key indicator of irreversible injury in cells. This attenuation of calcium overload also decreases the level of **B-cell lymphoma 2** (Bcl-2) and caspase-dependent apoptosis (Fig. 8) [93, 94, 95, 96]. Apoptosis is generally reported as very important in the process of cell death that claims numerous neurons within the cerebral ischemia region. Therefore, HM such as Chuanxiongzine of BHD and DCH with antiapoptotic capabilities, attenuate the level

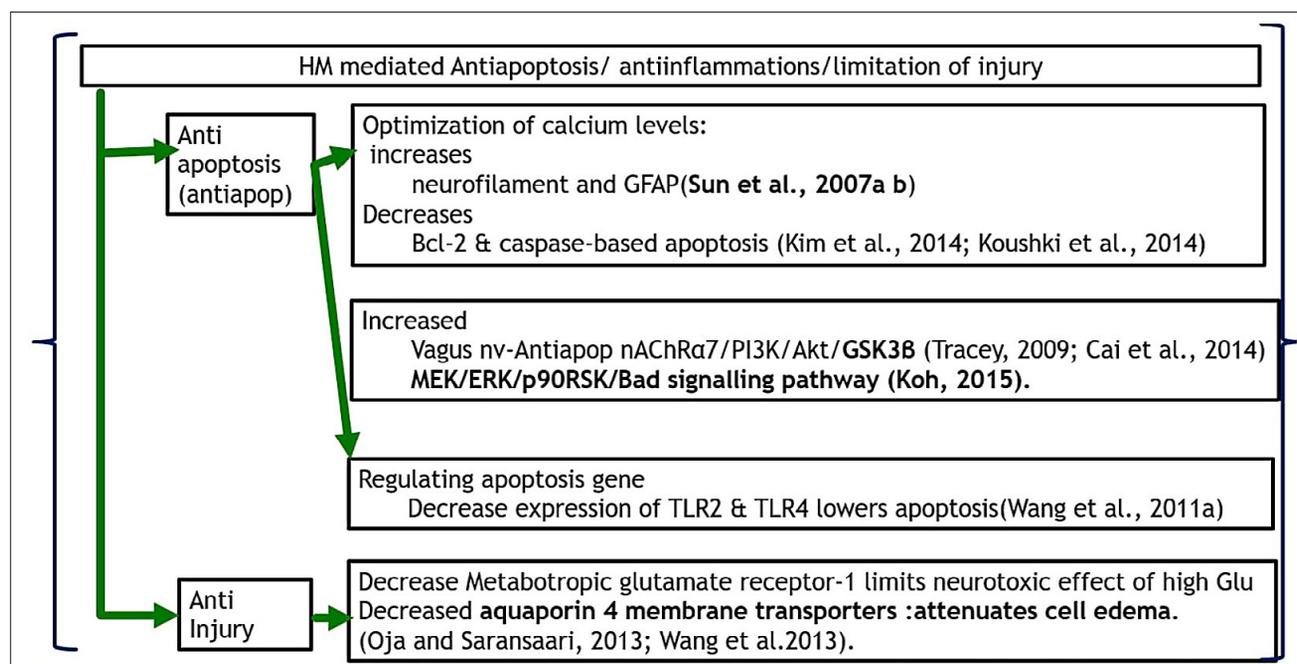


Figure 8. Molecular targets involved in the herbal medicine treatment for stroke (MTHM) via anti- inflammation and / or anti apoptosisgenesis

of cerebral ischemic reperfusion injuries that manifests as apoptosis [16, 93, 94, 95, 96, 97, 98].

Toll-like receptor (TLR)/myeloid differentiation factor 88 (MyD88). The level and rate of apoptosis is dependent in part on the level of expression of Toll-like receptor (TLR) / myeloid differentiation factor 88 (MyD88). Absence / attenuation of expression of specific types of TLR, such as TLR2 and TLR4, attenuates the degree to which cells malfunction and subsequently die via apoptosis after Ischemia /reperfusion injury [99]. Thus, the HM, such as Paeoniflorin, which attenuate the expression of TLR/MyD88 is a potent anti-apoptotic remedy that can suppress the level of neuronal death following cerebral ischemia.

Glutamate and toxic neural effect. Glutamate, an excitatory amino acid neurotransmitter is a promoter of toxic effect on neural cells by its activity on metabotropic glutamate receptor-1 when it occurs at very high levels in the extracellular spaces [100, 101, 102]. Therefore, regulation of the level of the activity of extracellular glutamate within an optimal non-toxic limit either by downregulating its glutamate receptor-1 or other means are putatively good mechanism utilized by some herbal medicines, such as **Ferulic acid (FA)**, in order to eliminate the neurotoxic effects of the glutamate (Fig. 8).

Aquaporin. Herbal remedies such as BYHW for treatment of cerebral ischemia reperfusion injury that attenuate the expression of aquaporin 4 with associated decreased propensity for cellular edema, have also been reported (Fig. 8) [50].

Pro-inflammatory cytokines. Pro-inflammatory cytokines, such as IL-1 β and TNF- α , can stimulate the detrimental activity of Nuclear factor kappa B (NF-Kb) with nuclear translocation for upregulating the expression of phospho-NF- κ B p65, promoting **NF- κ B inhibitory factor α (I κ B α)** degradation and heightening of the expression of **Protein P53** (p53) [33, 103]. These are well-known modulators of apoptosis (neuronal apoptosis) as well as structural brain injury (33, 103). These pro-inflammatory cytokines have also been reported to facilitate the degradation of the microenvironment around the nascent neuron with associated injuries that cumulatively shorten the life span of the neuron [104]. Herbal medicine, such as **Paeoniflorin (PF)**,

that attenuate the degradation of the microenvironment of newly-formed neurons, as well promote anti-apoptotic and anti-inflammatory effects, have great potential in the treatment of stroke (Fig. 8).

Phosphatidylinositol 3-kinase (PI3K)/ Akt/ GSK3 β anti-apoptotic pathway. The role of the the autonomic nervous system in regulating inflammation through the activity of the neural circuits on immune cells has been reported in the literature. It entails the activation of an nAChR α 7 anti-inflammatory pathway, which itself was recruited by the vagus nerve that is stimulated by circulating pro-inflammatory cytokines abintio (Fig. 8) [105, 106, 107]. Nevertheless, the increased activity of nAChR α 7 has been reported to facilitate neuronal recovery from cerebral ischemic injury). The activity of nAChR α 7 is mediated by the anti-apoptotic – phosphatidylinositol 3-kinase (PI3K)/ **Protein kinase B** (Akt) / **Glycogen synthase kinase 3 beta** (GSK3 β) pathway, which is poles apart from the phosphatase and tensin homolog (PTEN)- ROS production pathway. The activity of the nAChR α 7 receptor is also mediated by the proliferation-promoter β -arrestin mediated activation of Src pathways (Fig. 8) [108, 109, 110, 111]. PF is the main HM that shows great promise in modulating the apoptotic effects of PI3K.

MEK/ERK/p90RSK/Bad signalling anti-apoptotic pathway. The **Mitogen-activated protein kinase kinase** (MEK) / **extracellular signal-regulated kinases** (ERK) / **90 kDa ribosomal s6 kinases** (p90RSK) /Bad signalling pathway have been identified as a molecular cascade of events that prevent cerebral ischemic cell injury and death [112]. The HM of note that acts via this pathway is the FA.

Anti-coagulation / fibrinolysis effect. Attenuation of the aggregation of platelet, the levels of endothelin and thromboxane A₂, as well as the formation of a thrombus are central factors and/or mechanisms through which herbal medication protect against, as well as promote recovery from cerebral ischemic injury (Fig. 9) [113, 114, 115]. Increased fibrinolysis within the blood through the activity of endogenous tPA, as well as attenuation of the level of plasminogen activator inhibitor-1, have been experimentally identified as appropriate techniques used by herbal medicine, such as Huatuo Zaizao pill (HT) in ameliorating the level of injury following cerebral ischemia [116].

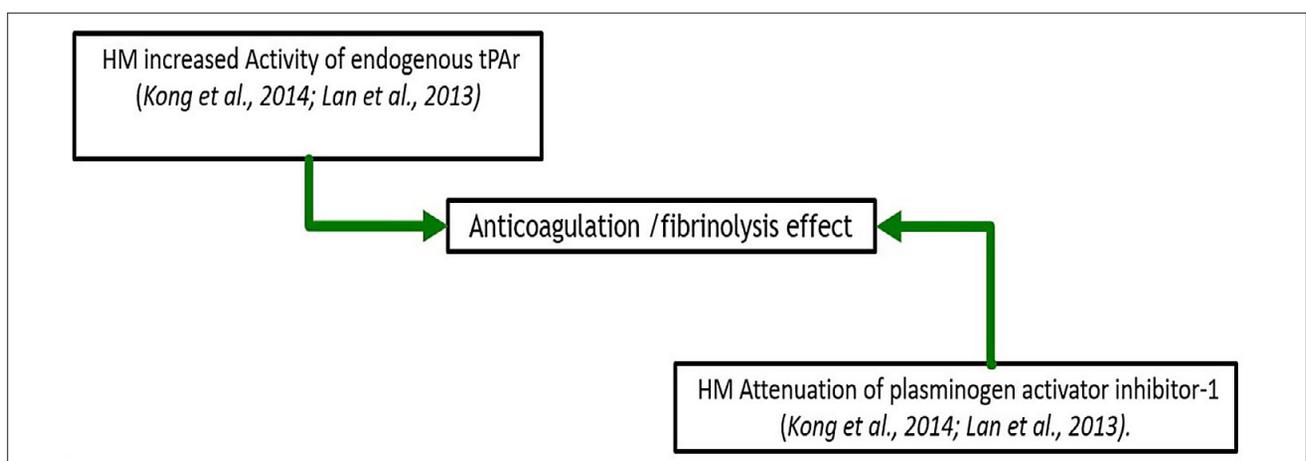


Figure 9. Molecular targets involved in the herbal medicine treatment for stroke (MTHM) via anti-coagulation

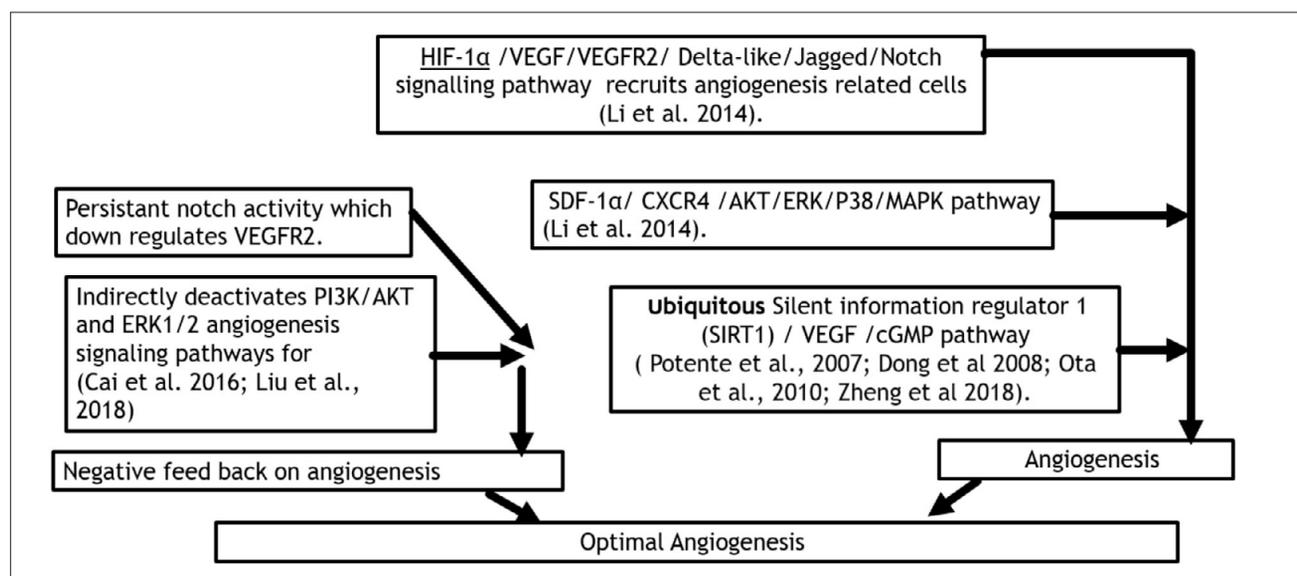


Figure 10. Molecular targets involved in the herbal medicine treatment for stroke (MTHM) via angiogenesis

Angiogenesis. There are a number of pro-angiogenesis cytokines that participate in neo-angiogenesis which include the SDF-1 α a ligand for the CXCR4 receptor, as well as the VEGF ligand for VEGFR. SDF-1 α recruits pro-angiogenic cells that manifest a prominent CXCR4 receptor, such as in the cells from HSCs, EPCs and smooth muscle cell progenitors, while VEGF binds to its receptor to mediate angiogenesis [117]. These two systems, SDF-1 α /CXCR4 and VEGF/VEGFR2, for recruiting angiogenesis related cells are reportedly parallel [117]. Rodent models of cerebral ischemia have been shown to have an up-regulation of SDF-1 α /CXCR4 axis with associated activation of **AKT, ERK, and P38** mitogen-activated protein kinase (MAPK) signalling pathways in the ischemic region of the brain, as well as increased angiogenesis and neurogenesis [118]. Increased activity of HIF-1 α , which primarily promotes activity of the VEGF / Delta-like/Jagged/Notch signalling pathway for angiogenesis, have been reported following treatment of stroke with herbal medicine (Fig. 10) [118]. Improvement in the activation of protein kinase B (AKT)/mammalian target of rapamycin (mTOR)/ hypoxia-inducible factor-1 alpha (HIF-1 α) pathway, as well as ERK1/2 molecular cascade, has also been documented in the literature and associated with the production of VEGF [117]. The outcomes, following the activation of these pathways include increase vascular density in *in vivo* studies, as well as increased BMEC migration and tube formation in *in vitro* studies [117].

The binding of VEGF on VEGFR activates tyrosine kinases with a number of downstream activities that promote angiogenesis [47, 117, 119, 120]. The VEGF cascades also promote the production of Delta-like 4 (Dll4) which is a specific endothelial ligand for Notch1 receptor in the arteries, and this component of the Notch signalling specifically promotes lumen morphogenesis in the next 1h suppression of the migration and proliferation of endothelial progenitor cells (EC) by down-regulating VEGFR, as well as indirect deactivation of the PI3K/AKT and ERK1/2 signalling pathways, also associated with the decline in the Notch signaling [121, 122]. This feedback activity helps militate against the detrimental effect of unchecked angiogenesis such as vascular malformations and hemorrhage [117, 123].

The key HM involved in optimizing angiogenesis include *Angelica gigas* NAKAI (AG) and FA.

The ubiquitous nature of silent information regulator 1/ sirtuin 1 (SIRT1) implicates it in many cells for a number of processes, such as inflammation, oxidation-stress, apoptosis, myogenesis and energy metabolism, due to its ability to interact with a wide range of transcription factors [124, 125, 126]. Increased activity of the SIRT1/ Vascular endothelial growth factor (VEGF) / Cyclic Guanosine Mono Phosphate (cGMP) pathway is a key pathway that plays a vital role in promoting angiogenesis, as reported in preclinical experimental studies with some HM such as BHD (Fig. 10) [16, 127, 128, 129]. Experimental evidence suggests that the level of VEGF begins to rise hours after cerebral reperfusion following an ischemic episode, and the rise in VEGF level plays a significant role in preventing cerebral damage during the delayed phase [128].

The activity of VEGF commonly occurs in tandem with the activity of Ang-1 which is essential for maturation and stabilization of blood vessels under either physiologic or pathologic conditions [130, 131, 132, 133, 13, 135]. It is also one of the ligands for tyrosine receptors (Tie-2) and the activation of this receptor leads to down-regulation of inflammatory responses associated with angiogenesis. It also promotes the expression of intracellular adhesion molecules, such as ICAM-1, **Vascular cell adhesion protein 1** (VCAM-1) and **endothelial-leukocyte adhesion molecule 1** (E-selectin) in endothelial cells during brain inflammation [23]. Generally, increased expression of vascular endothelial growth factor (VEGF), Ang-1, as well as late onset of increase in F1K1 expression had been reported in stroke studies that examined these angiogenesis-related proteins (ARP) protein (Fig. 9) [19, 136]. Herbal medicines such as *Angelica gigas* NAKAI (AG) and FA that modulate the expression and activities of ARP have shown promising results in the preclinical treatment of stroke.

Metabolism. A putative mechanism of action involving multi-target herbal medicinal products relies on the modulation of metabolism in the neuronal / glial cell pair following cerebral injury. Increased **N-Acetylaspartate (NAA) / Creatine (Cr)**

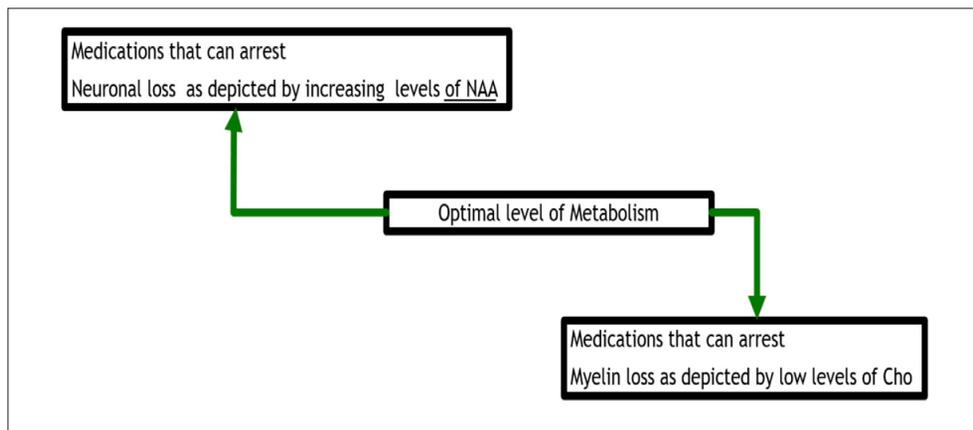


Figure 11. Molecular targets involved in the herbal medicine treatment for stroke (MTHM) via optimization of metabolism

ratio, as well as decreased Choline (Cho) / creatine (Cr) ratios were common in the initial time points after stroke among models receiving herbal medicine treatment [137] (Fig. 11). NAA metabolites depict the level of viability of neuron, while the Cho metabolite is mainly composed of compounds found in the cell membrane and depicts the level of metabolism (myelinization, proliferation and membrane function) in the supporting glial cells [137]. Attenuation of the levels of NAA suggests a high level of neuronal loss, while an elevated level of Cho is highly suggestive of dissolution of myelin as well as astrocytic gliosis [137, 138, 139, 140]. Therefore, HM such as *Xiaoshuan* enteric-coated capsule and BYHWD which are geared towards optimization of metabolic capabilities of neurons, have a strong promise for attenuating the detrimental effect of cerebral ischemia injuries (Fig. 11).

CONCLUSIONS

There is a dearth of clinically approved medications for the management of stroke, and those available are successful only in a few patients. The current review had successfully highlighted the efficacy of the HM, such as BHD, FA and HZP, and in ameliorating post-ischemic pathophysiology, which is an addition to the widely-held belief among researchers that they are best suited for preventive application. In addition, the multi-target molecular action of HM identified in this review may present clues for the development of more stroke medications based on its diverse approaches in ameliorating the pathophysiology of stroke. Therefore, the current review promotes awareness of the potential supportive role for HM which adds to the established available definitive treatment options for stroke, as well as providing a molecular compendium for potential druggable targets for stroke treatment.

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