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Experimental models and plant-based therapy for experimental cerebral ischemia (Review)

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Abstract. Cerebral ischemia is a leading cause of mortality worldwide. Available treatments are mainly thrombolytic agents for restoring blood flow to the brain. However, this approach has a very narrow treatment window. Despite extensive research, there is still a need for further investigations to identify and develop novel treatment approaches. The present review aimed to summarize and discuss evidence from the literature regarding the best models with which to study cerebral ischemia and the available herbal sources that may provide potential treatment strategies for cerebral ischemia. The present review was based on research published between 1990 and 2020. Herbal remedies provide a promising research area that warrants further attention from researchers in the field. Different models have been used to investigate the pathophysiology of cerebral ischemia/reperfusion, and to examine various treatment approaches. The plant kingdom is rich in various phytochemicals with neuroprotective functions. From the literature search performed herein, it can be concluded that middle cerebral and bilateral common carotid artery occlusion models are the most convenient, cost-effective and easily reproducible models. A number of plants, particularly those from Southeast Asia, have used for cerebral ischemia research; however, many more need to be investigated, particularly plants from Africa.

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Abbreviations: BBB, blood-brain barrier; BCCAO, bilateral common carotid artery occlusion; CCAs, common carotid arteries; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MRI, magnetic resonance imaging; SD, Sprague-Dawley; STAIR, Stroke Therapy Academic Industry Roundtable

Key words: cerebral ischemia, animal models, stroke, herbal medicine, cerebral ischemia/reperfusion

Contents

1. Introduction

Cerebral ischemia occurs when the blood flow to the brain is restricted, and it claims the lives of millions worldwide (1,2). In total, 16% of humans will have a stroke during their lifetime, with >15 million cases noted annually (1,3). Stroke is a complex disease with a narrow time window for therapeutic intervention to restore the blood supply and prevent permanent brain tissue damage (2). As a result, currently available strategies are considered inadequate (2). Therefore, there is a need for further research in order to understand the pathophysiology of the disease and to identify techniques that can reduce its severe complications (2).

Clinically relevant models are essential for cerebral ischemia research. These models should be clinically relevant and reproducible to aid in the understanding of the pathophysiology of ischemic stroke, as well as to function as a platform for the development of novel therapeutic approaches for stroke treatment.

For a number of years, plants and natural remedies have been the primary tool for folk medicine. Medicinal plants provide a cost-effective source of drugs with significant therapeutic benefits and few side-effects in comparison to commercial synthetic drugs. Herbal remedies may provide a source of novel compounds that may present novel therapeutic tools for cerebral ischemia and stroke. However, with the variants of models of cerebral ischemia, the literature lacks the link between the efficacy of the plant and the model used, which may represent a possible strategy with which to understand the mechanisms of these natural remedies.

The present review aimed to summarize and discuss the literature for data related to animal models utilized in the

research of cerebral ischemia, along with the herbal-based treatment approaches used in cerebral ischemia, in order to draw a full picture of the model used for treatment. The present review also aimed to illustrate the possible association between the natural ingredient in question that proved effective and the models of cerebral ischemia.

2. Data collection methods

Search strategy. Following the guidelines of Preferred Reporting Items For Systematic Reviews And Meta-Analyses (PRISMA) (4), the present review was conducted. The present review aimed to illustrate the data from peer-reviewed original articles on cerebral ischemia phytotherapy or from studies using *in vivo* models of cerebral ischemia. A search was conducted to obtain targeted articles through PubMed (https://pubmed.ncbi.nlm.nih.gov/) using the following keywords: 'Cerebral ischemia and herbal medicine'. The search span was between 1990 and 2020. In addition, the results were restricted to studies in the English language.

Selection criteria. The inclusion criteria were as follows: i) Only original studies between 1990 and 2020 were included in the present review; ii) any original article that assessed or used herbal extracts as a treatment approach for animal models of cerebral ischemia. On the other hand, the exclusion criteria were expanded to the following: i) Articles that reported the combination of plants as a formula/recipe; ii) research that was not published in the English language and were between 1990 and 2020; iii) case reports, review articles, or any secondary publications.

Data extraction. Search results were imported into Endnote X8 (Thompson Reuter) for the deletion of duplicates. The references were then screened by 3 reviewers, independently, using the eligibility criteria. The included articles were then reviewed, and data extraction was performed by 3 independent reviewers. Any disagreement in the extraction steps was raised to the supervisor to reach a consensus.

Risk of bias for individual studies. The included articles were investigated through the 'The Cochrane Collaboration's tool for assessing the risk of bias' (5). Any disagreements were discussed between authors to reach a consensus.

3. Search results

Search results and study characteristics. The search revealed 830 records; following title/abstract screening, 370 articles were selected. Following the screening of the full text of the articles, 52 studies were included in the present review.

Using the Cochrane risk of bias tool, we were uncertain of the bias regarding domains 4, 5 and 6 (5). However, most of the included studies showed a low risk of bias in the other domains.

Pathophysiology of cerebral ischemia. Ischemic stroke accounts for approximately 90% of all stroke cases in humans, followed by intracerebral hemorrhage (9%) and subarachnoid hemorrhage (3%). Ischemic stroke occurs due to the blockage of the middle cerebral artery (MCA). Cerebral tissue hypoxia and ischemia follow within minutes, leading to neuronal cell death and permanent damage to the brain (6). Thrombolytics and the rapid restoration of the blood supply remain the only treatment options with which to prevent further neuronal damage and decrease disability (6).

Ischemic damage to both white and grey matter causes permanent damage to brain tissue (7-9). Chronic cerebral hypoperfusion causes microglia/astrocyte activation, matrix metalloproteinase stimulation, blood-brain barrier disruption and endothelial abnormalities (10-12). Chronic cerebral hypoperfusion generates neuroinflammation, oxidative stress and apoptosis of the oligodendroglia (10-12). Aging, diabetes, atherosclerosis and hypertension are the most common risk factors that lead to chronic cerebral hypoperfusion (10-12).

In vitro models of cerebral ischemia. Testing different treatment approaches on human cells *in vitro* provides highly valuable, cost-effective and high-throughput systems for studies on stroke (13,14). Although *in vivo* models are preferred in cerebral ischemia, genetic differences and structural variations, as well as molecular differences exist, which renders clinical translation problematic (13); therefore *in vitro* models are still critical for the understanding of the molecular mechanisms of the disease. With the introduction of new technologies, there is an excellent opportunity for the development of *in vitro* systems to model stroke and improve drug discovery (15). For a full review of the *in vitro* models of cerebral ischemia, please see the study by Holloway and Gavins, 2016 (15).

Animal models in cerebral ischemia

Brain structure and function: Humans vs. animals. Although there are apparent differences between the human brain and the brains of other species, animal studies are critical in translational research. The debate continues as to whether these differences render the use of animal models in stroke studies irrelevant to the clinical application (16). These differences are evident in infarct localization (16). Another significant difference is the amount of white matter in the brain. The white matter accounts for 60% of brain tissue in humans, compared to 35% in dogs, 20% in rabbits, 15% in rats and only 10% in mice (17). This white matter difference poses a problem, as the ischemic damage of the white matter is a key player in the pathophysiology of stroke in humans (18).

Small vs. large animal models in cerebral ischemia. A number of animal species are used to investigate the mechanisms underlying cerebral ischemia (19-22). Different methods are used to generate cerebral ischemia, such as bilateral common carotid artery (CCA) occlusion (BCCAO) in rats (19), bilateral CCA stenosis (20), or asymmetric CCA surgery in mice (21), and three-vessel occlusions (3VO) in primates (22). The pros and cons of these models are illustrated in Fig. 1.

The majority of the stroke preclinical studies are conducted using small animals, particularly rodents. These studies have assisted researchers in understanding the molecular and biochemical processes within the ischemic tissue (23), as well as in understanding the different aspects of the injury mechanisms (24). However, despite the ease of handling rodents and the cognitive impairment produced as a consequence of

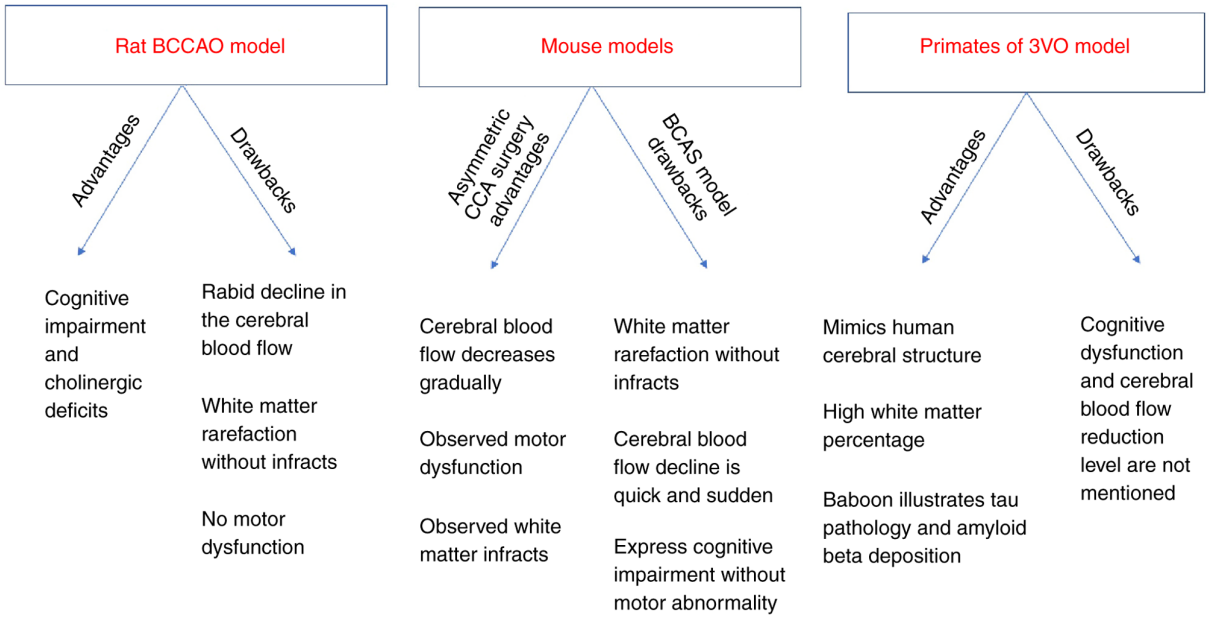

Figure 1. Small vs. large animal models of cerebral ischemia. BCCAO, bilateral common carotid artery occlusion; CCA, common carotid artery; BCAS, bilateral common carotid artery stenosis; 3VO, 3-vessel occlusion.

chronic cerebral hypoperfusion, no motor abnormalities or white matter infarcts have been generated (10,25,26).

The guidelines of the Stroke Therapy Academic Industry Roundtable (STAIR), dictate that clinical studies cannot be performed on humans before testing the new proposed pharmaceutical drugs on higher animal species (27-29). Nonetheless, the use large animal models in research is associated with various issues. One of these is the need for invasive surgery to generate and monitor ischemia and this causes a high mortality rate (24). Furthermore, large animal models are costly to maintain and are labor-intensive (24). Moreover, animal rights organizations have raised several concerns regarding the use of large animals (16).

One of the apparent advantages of using large animals, such as dogs, cats, pigs, sheep and primates (30) is that imaging is easier than with the use of small animals (31). It is also more suitable to monitor the physiology, e.g., blood pressure, and blood gases in large animals compared to small ones (24).

Another advantage is that the brain of large animals is similar to the human brain in terms of functionality and structure, i.e. gyrencephalic, while rodents have lissencephalic brains (24,32). In addition, the ratio of the neocortex to the basal ganglia and the volume of white matter indicate that large animals are closer to the human neuroanatomy (17,33,34). Furthermore, large animals are considerably similar to humans in several behavioral aspects, as well as sensorimotor integration (35). Primates exhibit similarities to humans regarding the cerebral structure and high white matter percentage (29,36). Furthermore, the baboon 3-vessel occlusion (3VO) model has exhibited a tau pathology and amyloid-β deposition similar to humans (37).

On the other hand, small animals, particularly rodents, are less costly to maintain compared to large animals (17,38). Moreover, the rat physiology and cerebrovascular anatomy are similar to those of humans (38,39), and mice have homogeneous genes, and as a result, genetic mutations are easily achievable to generate transgenic mice, commonly used to investigate the molecular pathophysiology of stroke (40,41). The small brain size in rats and mice may be seen as an advantage, as various fixation procedures can be performed for neurochemical and biochemical investigations (21).

Methods used to induce cerebral ischemia in animal models. Ischemic stroke in humans occurs, in most cases, due to middle cerebral artery (MCA) occlusion (MCAO) (42). A number of models are designed to restrict cerebral blood flow, permanently or transiently, either directly by occlusion of the MCA or indirectly by common carotid artery ligation (43). A craniectomy is required for the direct occlusion of one or more cerebral vessels, through ligation, clipping, with hooks, or electrocoagulation (44). This model resembles human cases in terms of transient and permanent ischemia (44). Cerebral ischemia (CI) in this approach affects most of the cortices (45) and protects the thalamic, hypothalamic, hippocampal and midbrain regions from damage, since it produces small infarcts, unlike other MCAO models (45). It also creates a high percentage of infarct size and neurological deficits. The visual confirmation of successful MCAO is achieved during the surgery, with subsequent reperfusion of the ischemic areas (46).

On the other hand, this technique can lead to the injury of the underlying cortex or rupture of vessels by drilling or electrocoagulation (46). Additionally, the intracranial pressure and blood-brain barrier (BBB) function are greatly affected, and, as a result, it requires superior surgical skills (46). All in all, this method is highly invasive, with several complications. Therefore, other models have been introduced to avoid such complications.

MCAO models. This technique is similar to 70% of cerebral ischemic strokes that occur in humans (33,47).

Koizumi *et al* (48) first established the intraluminal suture technique in 1986 in rats, and it was modified in the 1990s for use in mice (49). The intraluminal suture model offers two options: Transient ischemia with reperfusion or a permanent occlusion based on the time in which the suture is left in place (50). A craniotomy is not required in this model (50). A significant disadvantage is that it stimulates a larger infarction beyond the MCA zone to spread to the hippocampus and thalamus (51).

This model is reproducible in terms of primary ischemic injury and, consequently, cell death, blood-brain barrier (BBB) damage and glial activation (33,52). It is also appropriate for neuroprotection studies, due to the considerable existence of ischemic penumbra at the early stages post-occlusion (33). The infarct size in this model can be affected by the rat or mouse strain and the coating material for sutures. Strokes in spontaneously hypertensive rats (SHRs) are comparatively large and consistent in size. By contrast, in Sprague-Dawley (SD) rats, the infarcts are small and vary in size (53). An inadequate suture type may result in insufficient MCAO, leading to vessel rupture and subsequent subarachnoid hemorrhage (SAH). Silicone or poly-l-lysine coating suture is more adherent to neighboring vascular endothelium, compared to the uncoated suture, which leads to larger infarcts and minimizes inter-animal variability (54,55).

Unlike humans, following 60 min of MCAO, hypothalamic damage occurs (56). Following MCAO, hyperthermia occurs in rats and mice for at least 1 day due to hypothalamic ischemia (56,57). Approximately 12 h is needed in the transient MCAO mouse model for the damage to be repaired and for recovery to take place, which is a long therapeutic time, unlike stroke in humans (58). These two different mechanisms in the two MCAO models lead to varying results, which may be the reasons for the failure of neuroprotective agents in clinical experiments (58).

BCCAO. In several human cases of stroke, cerebral ischemia is a complication of thromboembolic conditions, such as carotid artery stenosis (59), atrial fibrillation (60) and heart failure (61). The BCCAO model is a relatively rapid and easy rat model that can be used to produce temporary or chronic cerebral ischemia (25). Rats usually exhibit white matter damage accompanied by cognitive impairments resembling those associated with stroke in humans. However, the affection of the visual pathways following the rat BCCAO compromises behavioral assessments. Therefore, C57/Bl mice have been used instead of rats, since the mouse model does not cause visual impairment and is, therefore, suitable for behavioral studies. This model is relatively easy to use, and training researchers on this mouse model decreases the mortality rate to <2%.

The bilateral common carotid artery stenosis (BCAS) model is a modification that causes carotid stenosis; the severity of cerebral hypoperfusion can be easily controlled by changing the diameter of micro-coils inserted in the carotid artery. This model is used worldwide and can be regarded as one of the most promising models of chronic cerebral hypoperfusion (10,20,62,63). This model mimics the white matter lesions induced by chronic cerebral hypoperfusion in humans (64).

Thromboembolic models. Thromboembolism is another cause of stroke. The thromboembolic clot model depends on the application route, the number and area of the clots (38). The thromboembolic clot can be spontaneous from autologous blood, which leads to vessel occlusion, causing infarcts (38). An advantage of this model is the spontaneous lysis of the clots, followed by reperfusion, which is similar to the case in humans (65).

An alternative method is the direct injection of thrombin into the MCA or internal carotid artery (ICA) to cause vascular occlusion (66). In this model, polymerized fibrin with a low number of cells and platelets are used to produce clots, while most of the human clots contain an accumulation of both platelets and fibrin, a deposition of neutrophils and monocytes and a high aggregation percentage of erythrocyte (67). In addition, the intravascular introduction of clots causes, in most cases, multifocal infarcts with noticeable variance in size and the localization of the lesion (68).

Another method with which to induce embolic stroke is by using microsphere/macrospheres to block blood flow. The major discrepancy with this model is that the fabricated spheres lead to permanent ischemia as they do not dissolve (65). An advantage associated with this model is the fact that the occlusion in rats may be postponed, while the animal is under monitor by PET or a magnetic resonance imaging (MRI) device (69).

Endothelin-1 model (ET-1). The reversible occlusion of the MCA can be achieved using a potent vasoconstrictor, such as endothelin-1 (ET-1) (33,38,70,71). A rapid blood flow reduction to the brain can be noted after the ET-1 injection, followed by reperfusion several hours later (72). Occlusion can be achieved through various mechanisms, directly by topical application onto the exposed MCA, or by intracerebral injection (73). A primary advantage of the injection method is the low invasiveness and the low mortality rate. Although ET1 is effective in rats, it is not as effective in mice (74). Another problem with this model is the variability of the lesion size (75).

Photo thrombosis model. This model requires an intravenous injection in rats or an intraperitoneal injection in mice (76,77) of a photosensitive dye, such as Rose Bengal or erythrosin B, followed by exposing the skull laser (78). Cerebral ischemia can be induced in specific brain regions depending on the purpose of the study. Once the dye is activated, reactive oxygen species (ROS) are generated, leading to endothelial damage, platelet activation and aggregation in pial and intraparenchymal vessels, forming thrombi (17). An advantage of this model is the rapid production of ischemic cell death (79). A further advantage is that it decreases the invasion and mortality rates. A significant disadvantage is that the ischemic injury is associated with early intracellular and extracellular edema formation (80), which differ from those observed in human stroke, as intracellular edema is the main indication of acute cerebral ischemia in humans (81).

In conclusion, as demonstrated through various sudies, there are various animal models, and associated techniques to produce cerebral ischemia/reperfusion, and each model has its advantages and weaknesses. The selection of the model should be based on the objectives and goal of the research study, bearing in mind that none of these models is identical to the human stroke pathophysiology.

Table I. Assessment of functional neurological deficits.

Test	Use	Method	Outcome
Rotarod test	The rotarod test assesses the rodent's motor coordination and balance, and in particular, detects abnormalities in the cerebellar function (84).	It requires putting the rodents on a cylinder, which rotates at different rates, and the time to fall is determined (85).	A significant decrease in the time the animal remains on the rod following stroke induction (86). Animal pre-training to balance on the rotarod before surgery is recommended.
Grip strength test	The grip strength test assesses the muscle force of rodents and detects any impairment in the limb strength (87).	The rodent grasps a bar or a grid attached to a force transducer to determine the strength of rodents while pulling it away (87).	Grip strength decreases significantly after stroke induction (88).
Wire hang test	As rodents tend to grasp any wire to avoid falling, the wire hang test is a cost-effective and easy method to evaluate the rodents' muscle performance.	The time that a rodent can hang, and this depends on several factors, including the weight of the rodent, its sex, muscle size, physical properties, and age (89).	In cerebral ischemia, muscle impairment is expected, and the ability to grasp the wire decreases (90). The main problem is that the recorded results are not always consistent (89).
The adhesive removal test	A sensitive technique to evaluate the rodent's sensorimotor deficiency (91).	The time to recognize the presence of adhesive tape placed on the rodent's forepaw and to remove it is recorded (91).	The prolonged time indicated a loss of sensory function.
Open field maze	The open field maze is one of the most widely used methods to investigate the rodents'behavior. It is a rapid and straight forwardmethod to evaluate the activity, both qualitatively and quantitatively (92,93).	The open field measures movements in an enclosure of varied shapes, either circular, square, or rectangular, with a surrounding fence to prevent rodents from escaping (93).	Studies have shown an increase in the crossed squares, rearing and grooming activities after ischemic stroke (148).
Water maze	The water maze method tests rodents' spatial understanding and learning (94).	The water maze test requires the placement of the rodent in a water pool, where there is a hidden platform under the water surface (94).	Memory and learning abilities are determined based on the time the rodent takes to reach the platform.
Modified neurological severity score (mNSS) points	The mNSS test (95) evaluates the motor, sensory, reflex and the beam balance on a scale from 0 to 18.		A score of 0 is normal and one of 18, is a maximal deficit.
Clark general and focal scales	The Clark scales divided into the general neurological scale and focal neurological scale (96).	The general test addresses the hair, ears, eyes, posture, spontaneous activity as well as the presence of epileptic behavior in the animals. The scores in the 6 areas are added to provide a total score ranging from 0 to 28. The focal test addresses the body and front limb symmetry, gait, climbing and circling behavior, and whisker response. The scores are added to provide a focal score ranging from 0 to 28.	The clinical score is highly correlated with the infarct volume.

Numbers in parentheses indicate relevant references.

Table II. Herbal extracts and their active ingredients used in rodent models of middle cerebral artery occlusion (MCAO).

Plant used	Doses/route	Active ingredients/action	Outcomes/results	(Refs.)
Artemisia absinthium L.	(100 mg/kg and 200 mg/kg, orally)	Antioxidant	Rat, ↓ brain oxidative stress and damage, and behavioral deficits	(97)
Eleutherococcus senticosus bark	Orally 3, 30 and 300 mg/kg twice at times of 0 and 90 min after reperfusion	Anti-inflammatory properties through the inhibition of COX-2 expression, microglia, and astrocyte expression.	Rat, vessel occlusion (4-VO)↓; hippocampal CA1 neuronal death at 300 mg/kg; ↓ COX-2, GFAP, and OX-42 in the hippocampal CA1 region	(115)
Embelia ribes Burm.	(100 and 200 mg/kg body weight; p.o.) for 30 days	Antioxidant	Rat, ↑ the grip strength activity, and GSH, GPx, GR and GST levels in hippocampus and frontal cortex; ↓ LDH levels in serum and TBARS levels in the hippocampus and frontal cortex	(113)
Erigeron breviscapus (Hand-Mazz)	i.p., 0.33 mg/kg	Breviscapine; targeting autophagy mechanisms	Rat, ↓ infarct volume, brain water content and neurofunctional deficiency	(101)
	Intraperitoneal injections of scutellarin (20 and 60 mg/kg)	Scutellarin; enhancing cellular antioxidant defense capacity	Rat, ↓ neurological deficits and brain infarct volume; ↑ endogenous antioxidant activity	(102)
Eriodictyol	Oral eriodictyol (1, 2 and 4 mg/kg) 30 min before pMCAO, 2 h after, and once daily for 5 days.	Inhibition of neuroinflammation	Rat, ↓ neuronal death, infarct area, neurological and memory deficits; ↓ MPO activity, TNF-α, iNOS, and GFAP expression	(114)
Fructus Chebulae	300 and 500 mg/kg orally	Inhibition of oxidative damages	Rat, ↓ cerebral infarct volume	(103)
Fructus Schisandrae	(10, 30 mg/kg, IP) 30 min before the onset of ischemia, and 2 h after reperfusion	Schisandrin B; inhibits Inflammation, and protects against metalloproteinase degradation.	Rat, ↓ infarct volumes; ↓ protein expression of TNF-a and IL-1b and degradation of MMP-2 and MMP-9 in ischemic hemispheres	(104)
Gastrodia elata (GE) Blume	IP 4-HBA (20 mg/kg) 1 h after MCAO	4-Hydroxybenzyl alcohol (4-HBA); antioxidant, anti-inflammatory, anti-excitotoxic, and anti-apoptotic effects	Rat, ↓ infarct volumes, motor impairments and neurological deficits; ↓ Zn^{2+}-induced cell death, ROS generation, and PARP-1 induction	(116)
Ginkgo biloba	45 mg/kg injected	EGb761; activating the Akt/CREB/BDNF pathway	Rat, ↑ behavior scores; ↑ phosphorylation of AKT, CREB and BDNF in the brain	(105)
Lavandula angustifolia	Orally once/day for 3 days before ischemia and once 2 h after ischemia	Lavender oil Antioxidant	Mice, ↓ neurological deficits, infarct size, MDA levels, carbonyl, ROS ↑ antioxidant capacity	(99)
Nigella sativa seeds	400 mg/kg, per orally for 7 days	Antioxidant, free radical scavenging, and anti-inflammatory properties	Rat, ↓TBARS levels ↑ glutathione, SOD and catalase levels	(117)
Panax ginseng	IP, 10 and 20 mg kg; orally, 100 mg/kg for 7 days before MCAO; oral, (0.5, 1, 5 or 10 mg/kg), every 3 days	20(R)-ginsenoside Rg(3); downregulation of calpain I and caspase 3; through Nrf2 pathways; ginsenoside Rb1	Rat, ↓ cerebral infarct volumes; ↓ calpain I and caspase-3 mRNA; mice, ↓ acute sensorimotor deficits; ↑ induction of Nrf2-downstream targets; mice, ↓ oxidative stress	(105-107)
Polygonum cuspidatum Sieb (knotgrass)	2.5, 5, 10 mg/kg tail vein injection 15 min after occlusion	Emodin-8-O-beta-D-glucoside; antioxidative effects	Rat, ↓ neurological deficits and the cerebral infarct area; ↑antioxidative; ↓ MDA level	(118)

Table II. Continued.

Plant used	Doses/route	Active ingredients/action	Outcomes/results	(Refs.)
Astragali radix	Intragastric calycosin (7.5, 15, 30 mg/kg)	Calycosin; antioxidant	Rat, ↓ neurological deficit and infarct volume; ↓ malondialdehyde (MDA), and reactive oxygen species (ROS); ↑the activity of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px); ↓ expression of 4-hydroxy-2-nonenal (4-HNE)	(119)
Pueraria radix	(25 and 50 mg/kg; intraperitoneally) 10 min before MCAO	Puerarin; inhibition of both HIF-1α and TNF-α	Rat, ↓infarct size; ↓ (HIF-1), (iNOS) and active caspase-3 protein expression; ↓ mRNA of TNF-α in ischemic regions	(120)
Scrophularia radix	2.4 g/kg-1	Regulating MAPK pathways	Mice, ↓ infarct volume, brain water content, (NO), (MDA), neurological deficits and LDH; ↑ antioxidant capacity	(121)
Salvia miltiorrhiza (Red sage) and cacao	Orally high dose (270 mg/kg) and low (27 mg/kg)	Antioxidants: Reducing the production of free radicals	Rat, ↓ ischemic cell death within the peri-infarct area; ↑ performance in routine motor and neurological tasks	(122)
Salvia miltiorrhiza (Red sage)	i.p. (15, 30 and 60 mg/kg); 30 and 60 mg/kg injected every 24 h for 5 days; i.p. 16 mg/kg	Magnesium lithospermate B; upregulation of p-Akt; vasodilation; ↓ platelet aggregation; ↓inflammation; sodium danshensu [3-(3,4-dihydroxyphenyl) lactic acid; inhibition of apoptosis by activating the PI3K/Akt pathway; tanshinones	Rat, ↓ neurological deficits; ↓ brain water content, glutamate levels, and cerebral infarct zones; rat, ↑ survival rate; ↓ infarct volume; ↓ neuronal death; ↓ number of apoptotic cells; ↑ ratio of Bcl-2/Bax; mice, 30% reduction in infarct size; improved neurological deficit	(100,123,124)
Scutellaria baicalensis (Chinese skullcap)	i.p. dose of 100 mg/kg	Baicalin; inhibits the expression of PAR-1	↑ Rat neurological function; ↓ cerebral infarct volume	(99)
Sophora flavescens Ait.	i.p. oxymatrine 120 mg/kg after MCAO	Oxymatrine; downregulation of 12/15-LOX, phospho-p38 MAPK and cPLA2	Rat; ↓ brain water content, and infarct volume; ↓ overexpression of 12/15-LOX, phospho-p38; MAPK and cPLA2	(125)
	0.5 mg kg, i.p.	Flavonoids kurarinone (45.5%) and sophoraflavone G (14.7%); inhibition of caspase-3 activation and reduction of DNA fragmentation	Rat; ↓ caspase-3 enzyme activity, and DNA fragmentation; ↓ cell apoptosis	(126)
Tripterygium wilfor0dii Hook.	Pre-treatment with TP (0.2 mg/kg) and DAHP (0.5 g/kg) by i.p. injection 0.2 mg/kg, IP 24 h before MCAO	DAHP and triptolide Activation of the PI3K/Akt/mTOR pathway and inactivation of the ERK1/2 pathway Inhibition of NF-κB activation	Rat, ↓ ischemic lesion volume, and neuronal cell death; ↓ astrocyte numbers, ↓ levels of Bax and caspase 3, ↑ NF-κB; ↑Bcl-2 expression;↑expression of PI3K, Akt, and mTOR; ↓ ERK1 and ERK2 phosphorylation (both studies) ↓ iNOS, COX-2, GFAP and NF-κB expression	(127,128)
Zizyphus jujuba and Silymarin	100, 250 and 500 mg/kg, p.o., or Silymarin (250 mg/kg, p.o.) for 3 days before MCAO	Amelioration of oxidative stress	Rat, ↓ neurological deficits, motor impairment, and cerebral infarction volume; ↓ oxidative stress	(129)

↓, decrease; ↑, increase.

Table III. Herbal extracts and their active ingredients used in other rodent models of cerebral ischemia.

Experimental model	Plant used	Dose/route	Active ingredients/actions	Outcomes/results	(Refs.)
Bilateral common carotid artery occlusion (BCAO) in rats	*Araucaria bidwillii*	Seven days pre-treatment with a bi-flavone fraction (BFR), 100 and 200 mg/kg	Biflavones; antioxidant	↑ Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH); ↓ lipid peroxidation (LPO) in various brain regions; ↓ neurological deficit and sensory-motor function	(112)
	Camellia sinensis	Green tea extract (0.5%) orally administered	Flavanol methylxanthines, theobromine, and theophylline; increased levels of hydrogen peroxide and inhibition of lipid peroxidation products in the ipsilateral hemisphere by the ischemia/reperfusion	↑ Hydrogen peroxide, and also inhibited the increased production of lipid peroxidation products; ↓apoptosis, ↓neuronal cell death	(110,111)
	Magnolia officinalis	10 and 30 mg/kg intravenous injection	Magnolol; downregulation of p38/MAPK, CHOP, and nitrotyrosine	↓ Infarct volume; ↓ inflammatory cytokines; ↓ production of nitrotyrosine, 4-hydroxy2-nonenal (4-HNE), inducible NO synthase (iNOS), various phosphorylated p38 mitogen-activated protein kinases, and different C/EBP homologs; ↓reactive oxygen species; ↑ expression of p-Akt and (NF-κB)	(130)
Global and focal cerebral ischemia/ reperfusion in rats and mice	*Panax ginseng*	*In vivo* Re (5, 10 or 20 mg kg 21, oral for 7 days, once a day) before occlusion	Ginsenoside	↓ MDA level AND mitochondrial swelling	(108)
	Momordica charantia	50, 100, 200 mg/kg at 30 min before cerebral ischemia, or 100 or 200 mg/kg, 30 min after cerebral ischemia	*M. charantia* polysaccharide (MCP); inhibiting oxidative stress	↓ NO, O2, ONOO and lipid peroxidation; ↓ activation of JNK3/c-Jun/Fas-L and JNK3/cytochrome c/caspase-3; signaling cascades in ischemic brains	(131)
	Ocimum basilicum	100 and 200 mg/kg	3,7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-methoxy-4-(2-propenyl) benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1, 8-cineole (0.288 mg/g); anticonvulsant, anti-inflammatory, and neurodegenerative; restoration of endogenous antioxidants; elevated brain glutathione content	↓ Infarct size and lipid peroxidation	(132-134)

Table III. Continued.

Experimental model	Plant used	Dose/route	Active ingredients/actions	Outcomes/results	(Refs.)
	Ocimum sanctum	Orally in doses of 200 mg/kg/day	Methanolic extract of OS leaves; anti-inflammatory, antioxidant, immunomodulatory and anti-stress properties	↑ SOD activity; prevented the rise in methane dicarboxylic aldehyde (MDA) levels	(135)
	O. europaea (olive oil)	Virgin olive oil consumption at 0.75 ml/kg/day	Monounsaturated fatty acids and polyphenols; oral low-dose (2.5 mg/kg of body weight) of hydroxytyrosol and a high-dose (10 mg/kg of body weight)	↓ Infarct volume, brain edema; ↑brain cerebroside levels	(136-138)
Spinal cord ischemia/reperfusion (I/R) injury in rats.	*Salvia miltiorrhiza* (red sage)	1, 10, or 50 mg/kg	Salvianolic acid B; ERK activation	↓ Spinal cord edema and infarct volume; ↑ motor function of the hind limbs; ↓ generation of oxidative products; ↑ antioxidant defense activities	(109)
Transient hippocampal ischemia in rats	*Melissa officinalis* (lemon balm)	100 μg/ml orally	Melissa oil; inhibition of HIF-1α and oxidative stress, followed by the inhibition of apoptosis	↓ Caspase-3 activity and malondialdehyde level; ↑ antioxidant capacity in the hippocampus; ↓ HIF-1α gene expression	(139)
Transient focal cerebral ischemia in mice	*Lavandula officinalis*	200 mg of lavender officinalis	Linalool-octanone, camphor, Caryophyllene, terpinen-4-ol, and flavonoids; decrease neurological deficit scores, infarct size, the levels of MDA, carbonyl and ROS, and attenuate neuronal damage, upregulated SOD, CAT, GSH-Px activities, and GSH/GSSG ratio	↓ Neurological deficit scores, infarct size, MDA, carbonyl and ROS, ↓ neuronal damage, antioxidant effects	(98,140)
Transient global ischemia in gerbils	Baicalin and Jasminoidin	Baicalin and jasminoidin, or nimodipine) were intravenously treated	Baicalin and jasminoidin; reduce neuronal damage in Gerbils hippocampus; lower MDA content, higher SOD, GSH, and GSH-PX activities	↓ Infarction area; ↓ lipid peroxidation; ↓ caspase-3	(141-143)
	Gastrodia elata		Free radical scavenging and antioxidant activity; increased expression of antioxidant genes	Protection of hippocampal neurons; ↓ infarct size in cortex and striatum	(144,145)
	Ginkgo biloba L.	Ginkgo biloba extract (37,5-150 mg/kg) orally	Ginkgo-flavone glycosides and terpenoids; antioxidant and free radical scavenging effects; decrease neurons against oxidative stress; decrease neuronal injury	Pre-treatment with *G. biloba* extract improved blood flow and reduced edema in the hippocampal region in a dose-dependent manner	(146,147)

↓, decrease; ↑, increase.

4. Assessment of functional deficits in cerebral ischemia models

Since stroke is associated with problems in the sensory and motor pathways, researchers have focused on studying the behavioral and cognitive aspects post-stroke (82). Various functional tests (Table I) are available for use in animals, including the Rotarod, the grip and string test, the wire hanging test, the adhesive removal test, the open field maze and the water maze test (83-95).

5. Herbal remedies as neuroprotective agents in cerebral ischemia

The MCAO model has been extensively studied with numerous natural herbal extracts. Cerebral ischemic injury results in the production of ROS, which cause lipid, DNA and protein oxidation, therefore causing cell damage and death (96). A number of these herbs possess antioxidant, anti-inflammatory and neuroprotective activity, such as *Artemisia absinthium* L. (97), *Lavandula angustifolia* (98), *Scutellaria baicalensis* (99) and several others. Their active ingredients exert significant neuroprotective effects and improvement in behavioral function when used before or after ischemic injury (98-100).

Chinese plants provide a rich source of herbal extracts for medicinal purposes. Some of these have been widely investigated in cerebral ischemia. Plants, such as *Erigeron breviscapus* (101,102) contain breviscapine, which is considered to target autophagy mechanisms, leading to a reduction in infarct size and functional improvements in rats. Scutellarin is another component of *Erigeron breviscapus*, which exerts a decrease in infarct size and functional improvement when injected into rats (102). Other Chinese plants include *Fructus Chebulae* (103) and *Fructus Schisandrae* (Chinese magnolia vine fruit) (104), which protect against metalloproteinase degradation. In rats, these fruits lead to a reduction in the expression of TNF-a and IL-1b, as well as reduction in the degradation of the metalloproteinases, MMP-2 and MMP-9, in ischemic hemispheres, which leads to a reduction in infarct size (104). *Panax ginseng* (105-107) reduces infarcts size in rats and mice and improves function through the downregulation of calpain I and caspase 3 (107), and the induction of Nrf 2 downstream targets (106). *Panax ginseng* has also been used in global and focal ischemic models (108); it causes a decrease in lipid peroxidation and mitochondrial swelling (108).

Salvia miltiorrhiza is a potent antioxidant that is used in MCAO (100) and in spinal cord ischemia/reperfusion injury in rats (109). It exerts a decrease in edema and infarct volume, as well as functional improvements in both models. Antioxidants, such as *Camellia sinensis* (110,111) and *Araucaria bidwillii* (112) cause functional improvements when used in global and focal ischemic models by inhibiting lipid peroxidation, and decreasing apoptosis and neuronal cell death.

Other herbal extracts and their active ingredients used in rodent models of MCAO are summarized in Table II (97-107,113-129). In Table III, the commonly used herbal extracts and their active ingredients used in other rodent models of cerebral ischemia are also listed (98,108-112,130-147). The possible mechanisms of action of each are highlighted.

6. Conclusions

Different *in vitro* and animal models are available for the study of cerebral ischemia/reperfusion injury. The pathophysiology of cerebral ischemia/reperfusion is complex, and animal models are superior, particularly when testing various treatment approaches. It is suggested that the MCAO and BCCAO models the most convenient, cost-effective and easily reproducible models. Herbal extracts and phytochemicals provide a wide variety of neuroprotective agents that may be of value to research in cerebral ischemia. Further investigations are required to identify the active ingredients of such plants, and further testing is warranted. The literature provides a wealth of knowledge regarding herbal medicine in cerebral ischemia research, mostly using plants from south-East Asia. Plants from Africa and other regions warrant further investigation as they provide attractive targets for the development of novel therapeutic drugs.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

NME, NS, EK, AA were involved in the conception and design of the study, and in the writing and revision of the manuscript. NE and NS were involved in the production of the figure and tables.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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