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Bioactive components of Chinese herbal medicine enhance endogenous neurogenesis in animal models of ischemic stroke

A systematic analysis

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Abstract

Background: Chinese herbal medicine (CHM) has been used to treat stroke for thousands of years. The objective of the study is to assess the current evidence for bioactive components of CHM as neurogenesis agent in animal models of ischemic stroke.

Methods: We searched PubMed, China National Knowledge Infrastructure, WanFang Database, and VIP Database for Chinese Technical Periodicals published from the inception up to November 2015. The primary measured outcome was one of neurogenesis biomarker, including Bromodeoxyuridine (BrdU), Nestin, doublecortin (DCX), polysialylated form of the neural cell adhesion molecule (PSA-NCAM), neuronal nuclear antigen (NeuN), and glial fibrillary acidic protein (GFAP).

Results: Thirty eligible studies were identified. The score of quality assessment ranged from 2 of 10 to 7 of 10. Compared with controls, 10 studies conducting neurobehavioral evaluation showed significant effects on bioactive components of CHM for improving neurological deficits score after ischemic insults ($P<0.01$ or $P<0.05$); 6 studies in Morris water-maze test showed bioactive components of CHM significantly decreased escape latency and increased residence time ($P<0.05$); 5 studies demonstrated that bioactive components of CHM significantly reduced infarct volume after ischemic stroke ($P<0.05$); 25 of 26 studies showed that bioactive components of CHM significantly increased the expression of BrdU and/or Nestin markers in rats/mice brain after ischemic injury ($P<0.05$, or $P<0.01$); 4 of 5 studies for promoting the expression of PSA-NCAM or DCX biomarker ($P<0.05$); 5 studies for improving the expression of NeuN biomarker ($P<0.05$); 6 of 7 studies for promoting the expression of GFAP biomarker in brain after ischemic stroke ($P<0.05$).

Conclusion: The findings suggest that bioactive components of CHM may improve neurological function, reduce infarct volume, and promote endogenous neurogenesis, including proliferation, migration, and differentiation of neural stem cells after ischemic stroke. However, evidences are supported but limited because only a few studies were available for each descriptive analysis. Further rigor study is still needed.

Abbreviations: BrdU = Bromodeoxyuridine, CAMARADES = Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies, CHM = Chinese herbal medicine, CNS = central nervous system, DCX = doublecortin, DG = dentate gyrus, GFAP = glial fibrillary acidic protein, MCAO = middle cerebral artery occlusion, NCAM = neural cell adhesion molecule, NeuN = neuronal nuclear antigen, NPCs = Neural progenitor cells, NSCs = Neural stem cells, PSA = Polysialic acid, SGZ = subgranular zone, SVZ = subventricular zone.

Keywords: bioactive components, chinese herbal medicine, experimental ischemic stroke, neurogenesis

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JHL, ZXC, and XGZ contributed equally to this work.

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1. Introduction

Neural stem cells (NSCs) are characterized as having properties of continuous proliferation and multiple differentiation potential. Since NSCs discovered in adult mouse striatum by Reynolds and Weiss in 1992,[1] intensive studies have indicated that neurogenesis can occur in the adult central nervous system (CNS).[2] Persistent neurogenesis mainly occurs in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) in the adult brain.[3–5] Neural progenitor cells (NPCs) generated from NSCs in both regions, confining in proliferation and differentiation into neurons or glia cells,[5,6] may offer an endogenous mechanism to brain repair and recovery from injury or disease.[7] Neurogenesis, which involves proliferation of NSCs/NPCs, differentiation of NPCs, and migration of neuroblasts, could be affected not only by multifarious physiological conditions including exercise,[8] enriched living conditions,[9,10] and aging[10,11] but also by various pathological conditions such as stroke,[12,13] psychosocial stress,[14,15] seizure,[16] and neurodegeneration.[17,18] Actively dividing cell population in the SVZ of adult rat is approximately 15% to 21%.[19–21] Previous study indicated that stroke substantially increased dividing SVZ cells up to 31% in mice model.[22] Though supplementing on survival and proliferation of intrinsic NSCs could assist to repair the damaged tissues, the efficacy of this supplementation has been shown to be limited.[23,24] Therefore, enhancing endogenous neurogenesis will have great potential application as a therapeutic strategy for CNS disorders. Neurogenesis markers, including Bromodeoxyuridine (BrdU), Nestin, doublecortin (DCX), polysialylated form of the neural cell adhesion molecule (NCAM), neuronal nuclear antigen (NeuN), and glial fibrillary acidic protein (GFAP) are widely used as the neuroregenerative development of proliferation, migration, and differentiation. BrdU, a synthetic thymidine analog used for measuring cell proliferation, incorporates DNA of dividing cells during the S-phase of the cell cycle.[25] Nestin, a class VI intermediate filament protein, is considered as a NSC/NPC biomarker during development of the CNS.[26] DCX is a microtubule-associated protein expressed by NPCs and immature neurons in embryonic and adult cortical structures, and used increasingly as a migration biomarker for neurogenesis.[27,28] Polysialic acid (PSA) is a linear homopolymer of alpha2–8-N-acetylneuraminic acid and the NCAM is the primary vector for it in vertebrates. PSA-NCAM participates in neural plasticity and neurogenesis, which is particularly considered toward cell migration.[29] NeuN, a homologue to sex-determining genes in Caenorhabditis elegans, is a neuronal nuclear antigen that is commonly used as a hallmark of neuronal differentiation during neurogenesis development.[30,31] GFAP, being described as one of the markers of astrocytic differentiation in vertebrates, is an intermediate-filament protein expressed uniquely in astrocytes and vulnerable to reactive gliosis that follows injuries to the CNS.[32]

Chinese herbal medicine (CHM) has been widely used to treat neurological disorders such as stroke,[33] Alzheimer disease,[34] Parkinson disease,[35] migraine,[36] depression, anxiety, and insomnia[37] in the young and/or the old. In addition, a wealth of active ingredients from herbs has been reported for their benefits to neural repair.[33] Of these bioactive components of CHM, studies showed that they have potential effects of promoting neurogenesis, neurite outgrowth, and synaptogenesis in ischemic stroke.[38,39] Systematic review of preclinical animal data could inform the planning and improve the likelihood of success of future clinical trials, identify where there is a need for further basic research, preclude unnecessary study replication, and contribute to both reduction and refinement in animal experimentation.[40] In addition, it might offer us with credible and solid new evidence on the neurogenesis effect in preclinical experiment to select the optimal requirements for drug administration for clinical trials. Thus, we conducted a preclinical systematic review of bioactive components of CHM as neurogenesis agent in animal models of ischemic stroke.

2. Methods

2.1. Ethical approval

All analyses were based on previous published studies; thus, no ethical approval and patient consent are required.

2.2. Database and literature search strategies

The databases, including PubMed, China National Knowledge Infrastructure, WanFang Database, and VIP Database for Chinese Technical Periodicals, were used for the literatures. To identify studies of bioactive components of CHM for neurogenesis after experimental ischemic stroke, we electronically operated each database from the inception up to November 2015. We also hand-searched a list of Chinese and English journals that may publish potentially eligible studies. Our search strategy included the following: ("stroke" OR "ischemia" OR "ischemic injury") AND ("neurogenesis" OR "neural regeneration" OR "nerve regeneration" OR "neuroregeneration" OR "proliferation") AND ("herbal" OR "Chinese medicine" OR "nature product" OR "active components" OR "bioactive compounds"). Chinese databases were also searched using the above search terms in Chinese.

2.3. Inclusion criteria

To prevent bias, inclusion criteria were prespecified as the following: experimental study of neurogenesis in ischemic stroke; animal model; the bioactive component of CHM was administered; control group was not administrated with any other CHM; the primary measured outcome was one of neurogenesis markers, including BrdU, Nestin, DCX, PSA-NCAM, NeuN, and GFAP.

2.4. Data extraction

The following details were extracted from each included study: the first author's name, publication year, category of bioactive components of CHM, type of models, the anesthetic method used during the induction of model; individual data for each study, including animal number, species, sex, weight; information on treatment including timing for initial treatment, type and method of treatment procedure, and duration of treatment; outcome measures including BrdU, Nestin, DCX, PSA-NCAM, NeuN, and GFAP, and the time point of outcome assessments; neurobehavioral assessment and infarct volume. We extracted data for mean value, standard deviation, and number of animals per group if appropriate. Meta-analysis was preformed if there were enough data of outcomes. Data extraction was performed by 2 independent authors.

2.5. Quality assessment

Based on the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) 10-item quality checklist[41] and the methodology

described by Macleod et al,[42,43] we modified "comparison" as the assessment of outcome in treatment and control groups after treatment with any bioactive components of CHM. The aggregate methodological quality score was calculated by applying a 10-item modified scale as following: publication in a peer-reviewed journal, control of temperature, random allocation to treatment or control, blinded induction of model, blinded assessment of outcome, use of an anesthetic without intrinsic neurogenesis activity, animal model (aged, diabetic, or hypertensive), performing a sample size calculation, compliance with animal welfare regulations, and a statement of potential conflicts of interest. One point was awarded for each item. We resolved any disagreement through discussion or consultation with corresponding author.

3. Results

3.1. Study inclusion

We identified 826 potentially relevant articles from the 5 databases, in which 27 records were excluded because of duplicates. After going through the titles and abstracts, we excluded 727 articles with at least one of following reasons: case report or review; not an animal research; and not the researches on neurogenesis of ischemic stroke. We then screened the remaining 72 articles, which reported the efficacy of bioactive components of CHM for ischemic stroke. Forty-two studies were excluded because of single Chinese herb or formulas or animal trials without disease model. Ultimately, 30 eligible studies were identified. Detailed information was shown in Figure 1.

3.2. Characteristics of included studies

The basic characteristics of included studies are shown in Table 1. Thirty studies included were published between 2003 and 2015.[44–73] The animal species included Sprague–Dawley rats for 21 studies,[44,45,47–53,56–58,61–65,68–70,73] Westar rats for 7 studies,[46,54,55,59,60,66,67] Mongolian gerbils and Kunming mice in each study.[71,72] The weight of rats varied from 180 to 375 g. Chloral hydrate was used in 17 studies to induce anesthesia,[44,45,48–54,57,58,65,69,70–73] pentobarbital was used in

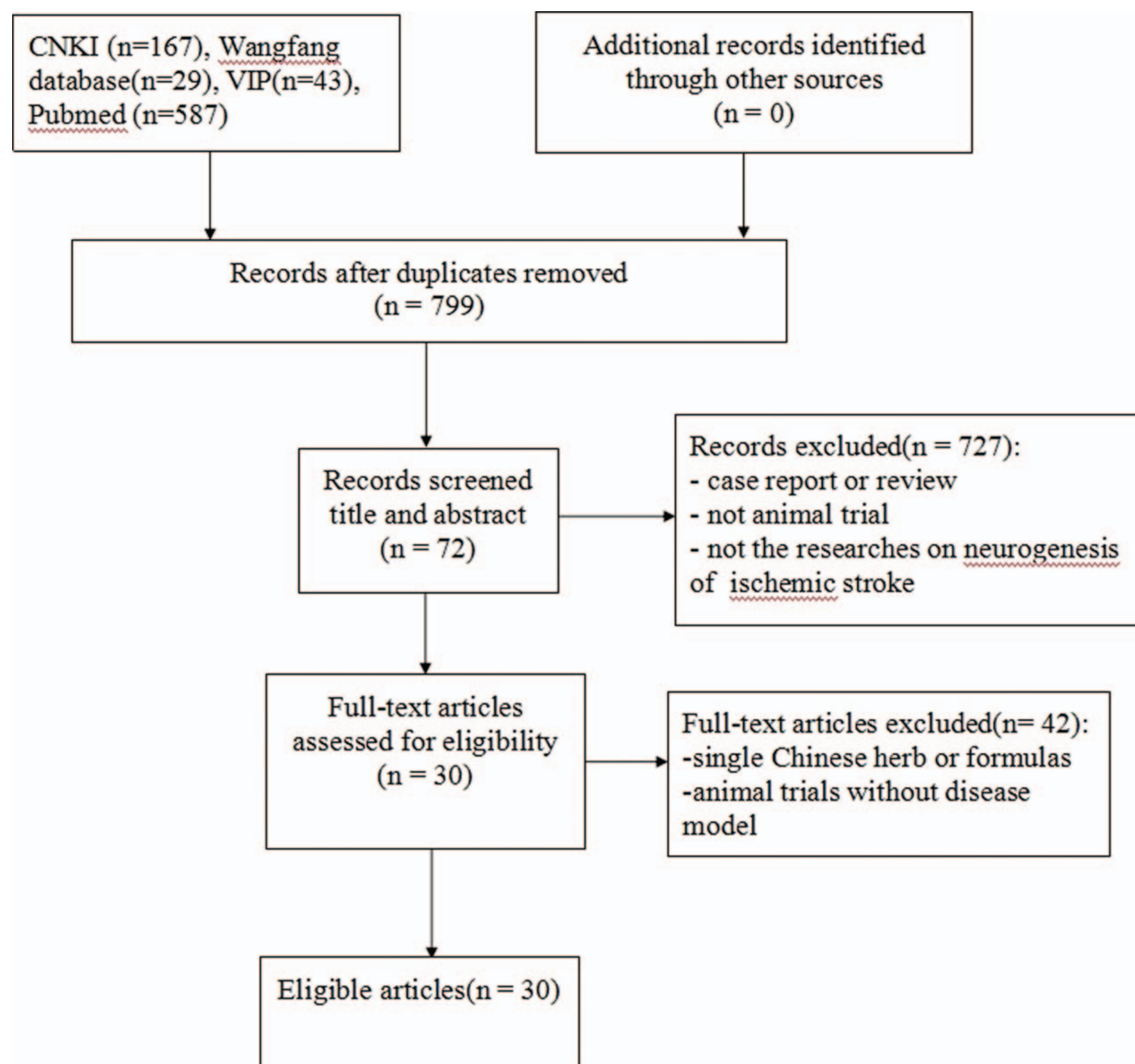

Figure 1. Flow diagram of the article selection for the study.

Table 1

The characteristic of included studies.

Study (years)	Type of herbal or bioactive compound	Species (sex, n)	Weight, g	Anesthetic	Model	Ischemic time (min)	Induced method	Intervention: Treatment group	Control group	Outcome measure	Treat group vs. model control group Mean ± SE (95% CI)	Intergroup differences
Zheng et al, 2013[44]	Bilobalide	SD rat (male, 12/12)	230–270	3.5% Chloral hydrate (0.1 mL/kg, i.p.)	MCAO	60	Modified Longa 1989	5, 10 mg/kg, i.g., once daily for 3 days	Sodium carboxymethyl cellulose	Neurological deficits score (Bederson test)	1. 1.41 ± 0.53 (1.11, 1.71) (5 mg), 1.23 ± 0.48 (0.96, 1.50) (10 mg) vs. 2.41 ± 0.52 (2.12, 2.70)	$P<0.01$ (5, 10 mg/kg)
										BrdU/MAP-labeled cells (ipsilateral cortex)	2. NR	$P<0.05$ (10 mg/kg)
Wang et al, 2014[45]	Gypenosides	SD rat (male, 9/11)	280–350	10% Chloral hydrate (3 mL/kg, i.p.)	MCAO	120	Modified Longa 1989	200, 400 mg/kg, i.g., once daily for 10 days	Saline	Neurological deficits score (Bederson test)	1. 1.64 ± 0.24 (1.50, 1.78) vs. 1 ± 0 (1, 1)	1. $P<0.05$ (400 mg/kg)
										Infarction volume	2. 10.06 ± 2.63% (7.75, 12.37) vs. 17.3 ± 0.12% (17.18, 17.42)	2. $P<0.05$ (400 mg/kg)
										BrdU-positive cells (SVZ)	3. 135.6 ± 12.45 (121.51, 149.69) vs. 63.33 ± 7.36 (55, 71.66)	3. $P<0.05$ (400 mg/kg)
										Nestin/DCX-labeled cells (SVZ)	4. 169.3 ± 10.98 (156.88, 181.72) (200 mg); 211.67 ± 25.18 (183.18, 240.16) (400 mg) vs. 82.67 ± 10.17 (71.16, 94.18)	4. $P<0.01$ (200, 400 mg/kg)
										5.Nestin/GFAP-labeled cells (SVZ)	5. 158.67 ± 4.67 (153.39, 163.95) (200mg); 275.33 ± 19.7 (253.04, 297.62) (400 mg) vs. 84.5 ± 15.5 (66.96, 102.04)	5. $P<0.01$ (200, 400 mg/kg)
Girbovan et al, 2015[46]	Resveratrol	Westar rat (male, 8/8/7 for 7 days groups, 11/12/10 for 85 days groups)	325–375	1.5% Halothane (inhalation)	Transient global ischemia	10	CCAOAC + VAIO	1, 10 mg/kg, i.p., once daily for 21 days	Saline	Escape latency (Morris)	1. NR	$P<0.01$ (7 days post-ischemia, 85 days post-ischemia)
										DCX/PSA-NCAM-labeled cells (DG)	2. NR	$P<0.05$ (7 days post-ischemia, 85 days post-ischemia)
He et al, 2015[47]	Total saponins of Panax notoginseng	SD rat (male, 6/6)	250–290	1% Sodium pentobarbital (50 mg/kg, i.p.)	Transient global ischemia	30	CCAOAC + VAIO	75 mg/kg, i.p., once daily for 1, 7 and 14 days	Saline	DCX/NeuN-positive cells (olfactory bulb)	NR	$P<0.01$ (7 days, 14 days)
Liu et al, 2015[48]	Ginsenoside Rd	SD rat (male, 6/6)	220–240	3.5% Chloral hydrate (i.p.)	MCAO	90	Modified Longa 1989	1, 2.5, and 5 mg/kg/, i.p., once daily for 3 days	Vehicle	Neurological deficits score (Bederson test)	1. NR	1. $P<0.05$ (5 mg/kg)
										Infarction volume	2. NR	2. $P<0.05$ (2.5, 5 mg/kg)
										BrdU/DCX-labeled cells (DG)	3. NR	3. $P<0.05$ (5 mg/kg)
										Nestin/GFAP-labeled cells (Striatum)	4. NR	4. $P<0.05$ (5 mg/kg)
Sun et al, 2013[49]	EGb 761	SD rat (male, 6/6)	220–250	10% Chloral hydrate (400 mg/kg, i.p.)	MCAO	60	Modified Longa 1989	0.525 mg/kg, LVI, once daily for 7 days	Saline	Neurological deficits score (Bederson test)	1. NR	$P<0.01$ (7 days)
										Infarction volume (7, 14 days)	2. NR	$P<0.05$ (14 days)
										BrdU/PSA-NCAM-labeled cells (DG region)	3. NR	$P<0.01$ (7 days) $P<0.05$ (14 days) 3. $P<0.05$
Liu et al, 2013[50]	Curcumin	SD rat (male, 5/5)	250–280	10% Chloral hydrate (350 mg/kg, i.p.)	MCAO	120	Modified Longa 1989	300 mg/kg, i.p., twice daily for 7 days	Corn oil	Brdu-positive cells (SVZ)	1. 50.60 ± 12.60 (42.79, 58.41) vs. 21.0 ± 5.23 (17.76, 24.24)	$P<0.01$ (7 days)
										Infarct volume	2. 67.80 ± 11.05 (60.95, 74.65) vs. 155.60 ± 11.26 (148.62, 162.58)	$P<0.01$ (7 days)
Cheng et al, 2013[51]	Curcumin	SD rat (male, 5/5)	250–280	10% Chloral hydrate (350 mg/kg, i.p.)	MCAO	120	Modified Longa 1989	300 mg/kg, i.p., twice daily for 7 days	Corn oil	Brdu-positive cells (SVZ)	1. NR	$P<0.05$ (7 days)
										BrdU/DCX cells (SVZ)	2. NR	$P<0.05$ (7 days)
Long et al, 2011[52]	Soy Isoflavones	SD rat (male, 6/6)	250–350	10% Chloral hydrate (300 mg/kg, i.p.)	MCAO	120	Modified Longa 1989	60 mg/kg, once daily for 37 days	—*	Neurological deficits score (Zeal longa)	1. 2.40 ± 0.52 (2.08, 2.72) vs. 2.90 ± 0.32 (2.70, 3.10)	$P<0.01$ (24 h)
										Brdu-positive cells (hippocampus)	2. 537 ± 60 (488.99, 585.01) vs. 416 ± 21 (399.20, 432.80)	$P<0.01$ (7 days)

Study (years)	Type of herbal or bioactive compound	Species (sex, n)	Weight, g	Anesthetic	Model	Ischemic time (min)	Induced method	Intervention		Outcome measure	Treat group vs. model control group Mean ± SE (95% CI)	Intergroup differences
								Treatment group	Control group			
Zhang et al, 2011[53]	Quercetin	SD rat (male, 5/5)	230–270	10% Chloral hydrate (350 mg/kg, i.p.)	MCAO	90	Nagasawa 1989	50 mg/kg, i.p., 3 times weekly until sacrifice	DMSO + saline	Neurological deficits score (modified Bederson/Zea longa)	1. 0.40 ± 0.13 (0.29, 0.51) vs. 1.33 ± 0.13 (1.22, 1.44)	$P < 0.01$ (7 days)
										Brdu-positive cells (SVZ)	2. 255.75 ± 22.91 (235.67, 275.83) (7d) vs. 152.50 ± 17.71 (136.98, 168.02); 310.41 ± 24.92 (291.21, 329.59) (14 days) vs. 251.25 ± 24.92 (229.41, 273.09); 320.60 ± 25.19 (298.52, 342.68) (21 days) vs. 194.33 ± 17.62 (178.89, 209.77)	$P < 0.01$ (7, 14, 21 days)
Zheng et al, 2011[54]	Ginseng total saponins	Wistar rat (male, 8/8)	250–300	10% Chloral hydrate (350 mg/kg, i.p.)	MCAO	Permanent	Modified Longa 1989	25 mg/kg/d, i.p., twice daily until sacrifice	Saline	Neurological deficits score (modified Bederson)	1. NR	$P < 0.05$ (14 days)
										Brdu-positive cells (SVZ)	2. NR	$P < 0.01$ (3, 7 days)
										BrdU+/NeuN+cells (ipsilateral infarct area)	3. NR	$P < 0.05$ (14 days)
										BrdU+/GFAP+cells (SVZ)		$P < 0.05$ (3, 7 days) $P < 0.01$ (14 days) $P > 0.05$
Gao et al, 2010[55]	Ginsenoside Rb1	Wistar rat (male, 4/4)	250–300	1% Pentobarbital sodium (30 mg/kg, i.p.)	MCAO	120	Modified Longa 1989	40 mg/kg i.p.	Saline	Neurological deficits score (modified NSS)	1. NR	$P < 0.05$ (1, 3, 5 days)
										Nestin-positive cells	2. NR	$P < 0.05$ (12 h; 1, 2, 3, 5, 10 days)
Xiao et al, 2010[56]	Tetramethylpyrazine	SD rat (male, 6/6)	180–220	2.5% Sodium pentobarbital (i.p.)	MCAO	120	Modified Longa 1989	10, 20, or 40 mg/kg, i.p., once daily until sacrifice	Saline	Infarct volume	1. NR	$P < 0.05$ (3 days; 10, 20, 40 mg/kg)
										Brdu positive cells (20mg/kg; SVZ)	2. 161.3 ± 26.70 (139.94, 182.66) (1 day) vs. 29.6 ± 3.70 (26.64, 32.56); 163.8 ± 14.30 (152.36, 175.24) (3 days) vs. 31.3 ± 3.70 (28.34, 34.26); 249.0 ± 27.8 (226.76, 271.24) (7 days) vs. 148.3 ± 19.1 (133.02, 163.58); 168.0 ± 29.9 (144.08, 191.92) (14 days) vs. 139.7 ± 29.1 (116.42, 162.98); 132.5 ± 22.8 (114.26, 150.74) (21 days) vs. 74.3 ± 34.2 (46.93, 101.67)	$P < 0.01$ (1, 3, 7, 14, 21 days)
										BrdU+/NeuN+cells (20mg/kg, ipsilateral infarct area)		$P < 0.05$ (21 days)
										BrdU+/GFAP+cells (20 mg/kg, ipsilateral infarct area)		$P < 0.05$ (21 days)
											3. 67.3 ± 5.8 (62.66, 71.94) vs. 21.9 ± 3.5 (19.10, 24.70)	
											4. 35. ± 3.1 (33.12, 38.08) vs. 29.8 ± 2.6 (27.72, 31.88)	
Yao et al, 2009[57]	Cornel iridoid glycoside	SD rat (male, 7/7)	250–270	10% Chloral hydrate (0.4 mL/kg, i.p.)	MCAO	90	Modified Longa 1989	20, 60, 180 mg/kg, i.g., once daily until sacrifice	Saline	Neurological deficits score (60, 180 mg/kg; modified NSS)	1. 7.17 ± 0.44 (6.90, 7.44) (7 days, 60 mg), 7.50 ± 0.27 (7.31, 7.69) (7 days, 180 mg) vs. 8.75 ± 0.37 (8.51, 8.99) (7 days); 4.22 ± 0.43 (3.95, 4.49) (14 days, 60 mg), 4.89 ± 0.35 (4.65, 5.13) (14 days, 180 mg) vs. 6.25 ± 0.45 (5.96, 6.54); 3.51 ± 0.46 (3.22, 3.80) (28 days, 60 mg), 3.88 ± 0.55 (3.50, 4.26) (28 days, 180 mg) vs. 5.5 ± 0.38 (5.25, 5.75)	$P < 0.01$ (7, 14, 28 days; 60 mg/kg)
										Brdu positive cells (20, 60, 180 mg/kg; SVZ)		
										The density of nestin-positive cells (20, 60, 180 mg/kg; SVZ)	2. NR	$P < 0.05$ (7, 14, 28 days; 180 mg/kg)
										BrdU+/NeuN+ positive cells (28 days; 20,	3. NR	

Study (years)	Type of herbal or bioactive compound	Species (sex, n)	Weight, g	Anesthetic	Model	Ischemic time (min)	Induced method	Intervention: Treatment group	Intervention: Control group	Outcome measure	Treat group vs. model control group Mean ± SE (95% CI)	Intergroup differences
										60, 180 mg/kg; ipsilateral striatum) BrdU+/GFAP+ cells (20, 60, 180 mg/kg; ipsilateral striatum)	4. NR	$P<0.01$ (7, 14, 28 days; 180 mg/kg) $P<0.01$ (14, 28 days; 60 mg/kg) $P<0.05$ (7 days; 60 mg/kg) $P<0.05$ (14, 28 days; 180 mg/kg) $P<0.05$ (28 days; 60 mg/kg) $P<0.01$ (14 days; 60 mg/kg) $P<0.05$ (180 mg/kg, 28 days) $P<0.01$ (60 mg/kg, 28 days) $P<0.05$ (60 mg/kg, 28 days)
Zhong et al, 2007[58]	Salvianolic acid B	SD rat (male, 6/6)	260–300	10% Chloral hydrate (0.35 mL/kg, i.p.)	MCAO	120	Longa 1989	1, 10 mg/kg, i.p., once daily until sacri-fice	Saline	1. Brdu-positive cells (7days; 1, 10 mg/kg; SVZ, SGZ)	1. 210 ± 11 (201.2, 218.8) (1 mg, SVZ), 300 ± 27 (278.40, 321.60) (10 mg, SVZ) vs. 125 ± 21 (108.20, 141.80) (SZV); 19 ± 2 (17.4, 20.6) (1 mg, SGZ), 47 ± 4 (43.8, 50.2) (10 mg, SGZ) vs. 16 ± 3 (13.6, 18.4)	$P<0.01$ (7 days; 1, 10 mg/kg; SVZ) $P<0.01$ (7 days; 10 mg/kg; SGZ)
Cui et al, 2007A[59]	Ginsenoside Rgl	Wistar rat (male 5/5)	250–300	—*	MCAO	Permanent	Longa 1989	20 mg/kg, i.p., twice daily until sacrifice	Saline	Brdu-positive cells (SVZ)	1. 14.1 ± 1.5 (12.79, 15.41) (1 day) vs. 11.7 ± 1.2 (10.65, 12.75); 28.8 ± 3.3 (23.77, 29.03) (3 days) vs. 21.3 ± 2.7 (18.93, 23.67); 35.10 ± 4.2 (31.42, 38.78) (7 days) vs. 26.40 ± 3 (23.77, 29.03); 21.6 ± 2.7 (19.23, 23.97) (14 days) vs. 16.8 ± 1.2 (15.75, 17.85)	$P<0.01$ (1, 3, 7, 14 days)
										BrdU/GFAP cells (SVZ)	2. NR	$P<0.01$ (14 days)
Cui et al, 2007B[60]	Ginsenoside Rgl	Wistar rat (male 5/5)	250–300	—*	MCAO	Permanent	Longa 1989	20 mg/kg, i.p., twice daily	Saline	Brdu-positive cells (SGZ)	1. 4.7 ± 0.5 (4.26, 5.14) (1 day) vs. 3.9 ± 0.4 (3.55, 4.25); 9.6 ± 1.1 (8.6064, 10.56) (3 days) vs. 7.1 ± 0.9 (6.31, 7.89); 11.7 ± 1.4 (10.47, 12.93) (7 days) vs. 8.8 ± 1.0 (7.92, 9.68); 7.2 ± 0.9 (6.41, 7.99) (14 days) vs. 5.6 ± 0.4 (5.25, 5.95)	$P<0.01$ (1, 3, 7, 14 days)
										Nestin-positive cells (SGZ)	2. 7.6 ± 0.8 (6.9, 8.3) (1 day) vs. 4.7 ± 0.3 (4.44, 4.96);	$P<0.01$ (1, 3, 7, 14 days)
										BrdU/nestin cells (SGZ)	15.8 ± 1.1 (14.84, 16.76) (3 days) vs. 10.4 ± 0.9 (9.61, 11.19); 27.3 ± 2.0 (25.55, 29.05) (7 days) vs. 21.1 ± 1.4 (19.87, 22.33); 13.2 ± 1.2 (12.15, 14.25) (14 days) vs. 6.2 ± 0.5 (5.76, 6.64) 3. 2.4 ± 0.7 (1.79, 3.01) (1 day) vs. 1.6 ± 0.5 (1.16, 2.04); 13.8 ± 2.6 (11.52, 16.08) (3 days) vs. 7.1 ± 1.6 (5.70, 8.50); 16.7 ± 3.1 (13.98, 19.42) (7 days) vs. 11.5 ± 2.4 (9.40, 13.60); 5.3 ± 1.3 (4.16, 6.44) (14 days) vs. 3.8 ± 1.1 (2.84, 4.76)	$P<0.01$ (1, 3, 7, 14 days)
Qi et al, 2007[61]	Ligustrazine	SD rat (male, 6/6)	260–300	—*	MCAO	120	Longa 1989	40 mg/kg, i.p., once daily until sacrifice	Saline	Brdu-positive cells (SVZ)	1.NR	$P<0.01$ (1, 3 days)

Study (years)	Type of herbal or bioactive compound	Species (sex, n)	Weight, g	Anesthetic	Model	Ischemic time (min)	Induced method	Intervention		Outcome measure	Treat group vs. model control group Mean±SE (95% CI)	Intergroup differences
								Treatment group	Control group			
Qiu et al, 2006A[62]	Ligustrazine	SD rat (male, 3/3)	230–260	Pentobarbital (40 mg/kg, i.p.)	MCAO	Permanent	Modified Longa 1989	80 mg/kg, i.p., once daily until sacrifice	Saline	1. Brdu-positive cells (SGZ)	1. 190.30±2.66 (187.29, 193.31) (7 days) vs. 119.48±3.57 (115.44, 123.52); 264.59±2.96 (261.24, 267.94) (14 days) vs. 212.05±7.06 (204.06, 220.04); 305.54±2.51 (302.70, 308.38) (21 days) vs. 147.84 ±0.64 (147.12, 148.56)	1. $P<0.01$ (7, 14, 21 days)
Qiu et al, 2006B[63]	Ligustrazine	SD rat (male, 3/3)	220–260	10 g/L Pentobarbital (40 mg/kg, i.p.)	MCAO	Permanent	Modified Longa 1989	80 mg/kg, i.p., once daily until sacrifice	Saline	1. Brdu-positive cells	1. NR	1. $P<0.01$ (7, 14, 21 days; cortex) $P<0.05$ (7, 14, 21 days; striatum)
Qiu et al, 2006C[64]	Ligustrazine	SD rat (male, 3/3)	220–260	10 g/L Pentobarbital (40 mg/kg, i.p.)	MCAO	Permanent	Modified Longa 1989	80 mg/kg, i.p., once daily until sacrifice	Saline	1. Brdu-positive cells (SVZ)	1. 180.13±3.87 (175.75, 184.51) (7 days) vs. 134.56±0.61 (133.87, 135.25); 203.72±2.61 (200.77, 206.67) (14 days) vs. 151.96±4.39 (146.99, 156.93)	$P<0.01$ (7, 14 days)
Hu et al, 2004[65]	Protoparaxotriol apo-nins	SD rat (male 5/5)	250–300	5% Chloral hydrate (6 mL/kg, i.p.)	MCAO	120	Modified Longa 1989	50 mg/kg, i.p., once daily until sacrifice	Saline	Brdu-positive cells (border zone of infarct)	NR	$P<0.05$ (7, 14, 28 days)
										BrdU/nestin cells (border zone of infarct)	NR	$P<0.05$ (7, 14 days)
Zhuang et a, 2012[66]	Salvianolic acid B	Wistar rat (male, 4/4)	200–250	Sodium pentobarbital (i.p.)	Transient global ischemia	6	CCAOAC+VAIO	50 mg/kg, i.p.; once daily for 4 weeks	Distilled water	BrdU-positive cells (SGZ)	1. NR	1. $P<0.05$
										Escape latency (Morris)	2. NR	$P<0.05$
										Time spent in target quadrant	3. NR	$P<0.05$
Zhuang et al, 2013[67]	Baicalin	Wistar rat (male, 3/3)	200–250	Sodium pentobarbital (i.p.)	Transient global ischemia	6	CCAOAC+VAIO	50 mg/kg, i.p.; once daily for 3 weeks	Saline	BrdU-positive cells (SGZ)	2. 3.8±4.3 (18.93, 28.67) vs. 12.9± 6.3 (5.77, 20.03)	$P<0.05$ (21 days)
										BrdU-NeuN positive cells (SGZ)	11.5±3.8 (7.2, 15.8)	$P<0.01$ (21 days)
										Escape latency (Morris)	vs. 4.8±2.6 (1.86, 7.74) 3. 20.05±10.35 (13.64, 26.46) vs. 38.23±24.29 (23.18, 53.28)	$P<0.05$
Li et al, 2012[68]	Puerarin	SD rat (female, 15/15)	240–260	—*	Transient global ischemia	10	CCAOAC+hypo-tension	i.p.; for 2 weeks	Saline	Escape latency (Morris)	1. 10.2±3.1 (8.63, 11.77) vs. 17.6± 5.7 (14.72, 20.48)	$P<0.001$ (14 days)
										BrdU-positive cells (SVZ)	2. 362±76.3 (323.39, 400.61) vs. 295±63.1 (11.77, 326.93)	$P<0.001$ (14 days)
Li, 2011[69]	Baicalin	SD rat (male, 5/5)	230±20	3.5% Chloral hydrate (1 mL/100 g)	Transient global ischemia	20	CCAOAC+hypo-tension	100 mg/kg, i.g., twice daily until sacrifice	Saline	Escape latency (Morris)	1. 49.38±14.64 (37.67, 61.09) (3 days) vs. 63.25±14.35 (51.77, 74.73); 32.82±12.47 (22.84, 42.80) (4 days) vs. 53.97±9.54 (46.34, 61.60); 19.41 ±6.37 (14.3, 24.51) (5d) vs. 46.37± 14.91 (34.44, 58.30)	$P<0.05$ (3, 4, 5 days)
										Residence time in the target quadrant		$P<0.01$
										Platform-cross number		$P<0.01$
										BrdU-positive cells (DG, SVZ)	2. 62.81±18.63 (47.90, 77.72) vs. 36.27±13.84 (25.20, 47.34) 3. 5.80±1.42 (4.66, 6.94) vs. 2.34± 0.67 (1.80, 2.88) 4. 6.8±2.79 (4.57, 9.03) (3 days, DG) vs. 6.4±2.34 (4.53, 8.27) (3 days, DG); 14.4±4.14 (11.09, 17.71) (7 days, DG) vs. 10.5±3.58 (7.64, 13.36); 11.0±3.58 (8.14, 13.86) (14 days, DG) vs. 7.6±2.51 (5.59, 9.61); 64.8±8.82 (57.07, 72.53) (3 days, SVZ) vs. 60.4±6.12 (55.04, 65.76); 126.2±13.47 (114.39, 138.01) (7 days, SVZ) vs. 80±4.82 (75.78, 84.22); 90±12.76 (78.82, 101.18) (14 days, SVZ) vs. 63± 10.09 (54.16, 71.84)	$P<0.05$ (3, 7, 14 days)

Study (years)	Type of herbal or bioactive compound	Species (sex, n)	Weight, g	Anesthetic	Model	Ischemic time (min)	Induced method	Intervention: Treatment group	Intervention: Control group	Outcome measure	Treat group vs. model control group Mean ± SE (95% CI)	Intergroup differences
Wang et al, 2009[70]	Astragaloside IV	SD rat (male, 5/5)	250–300	10% Chloral hydrate (350 mg/kg, i.p.)	Transient global ischemia	6	CCAOAC + VAIO	2, 4 mg/kg, i.p., once daily until sacrifice	Saline	BrdU-positive cells (2, 4 mg/kg; SGZ, CA1)	1. 41.50 ± 9.17 (32.51, 50.49) (2 mg, DG), 55.50 ± 7.78 (47.88, 63.12) (4 mg, DG) vs. 15.50 ± 7.05 (8.59, 22.41); 26.75 ± 6.40 (20.48, 33.02) (2 mg, CA1) 51.00 ± 14.14 (37.14, 64.86) (4 mg, CA1) vs. 5.50 ± 4.43 (1.16, 9.84)	$P < 0.05$ (7 days)
										BrdU/GFAP-positive cells (2, 4 mg/kg; SGZ, CA1)		$P < 0.05$ (7 days; 4 mg/kg; CA1)
										BrdU/MAP-2 positive cells (SGZ and CA1)		$P < 0.05$ (2, 4 mg/kg)
											2. 5 ± 2.45 (2.60, 7.40) (2 mg, DG), 6 ± 1.41 (4.62, 7.38) (4 mg, DG) vs. 4.5 ± 5.69 (−1.08, 10.08); 1 ± 0 (1, 1) (2 mg, CAI) 5.00 ± 1.41 (3.62, 6.38) (4 mg, CA1) vs. 0 ± 0 (0, 0);	
											3. 34.25 ± 5.61 (28.75, 39.75) (2 mg, DG), 35.25 ± 2.22 (33.07, 37.43) (4 mg, DG) vs. 23.00 ± 3.16 (19.9, 26.1); 7.25 ± 2.87 (4.44, 10.06) (2 mg, CAI), 26.25 ± 12.38 (14.12, 38.38) (4 mg, CAI) vs. 6.5 ± 5.92 (0.7, 12.3)	
Shen et al, 2003[71]	Ginsenoside Rg1	Mongolian gerbil (male, 4/4)	60–80	Trichloroacetaldehyde monohydrate (300 mg/kg, i.p.)	Transient forebrain ischemia	6	CCAOAC	5 and 10 mg/kg, i.p., once daily until sacrifice	Saline	1. BrdU-positive cells (5,10 mg/kg; SGZ)	1. 407.9 ± 56.3 (362.85,452.95) (11 days, 5 mg)	1.$P < 0.05$ (5 mg/kg, 7 days)
											311.2 ± 31.7 (285.84, 336.56) (11 days,10 mg) vs. 209.2 ± 29.2 (185.84, 232.56)	
												$P < 0.05$ (5, 10 mg/kg, 11 days) $P < 0.05$ (10 mg/kg, 21 days) $P < 0.05$ (5 mg/kg, 40 days)
Hou, 2007[72]	L-3-n-butylphthalide	Kunming mouse (male, 6/6)	30–35	10% Chloral hydrate (0.35 mL/100 g)	MCAO	90	Modified Longa 1989	10, 20 mg/kg, i.p., once daily until sacrifice	Saline	Neurological evaluations based on 6 tests	1. 12.7 ± 1.0 (11.90, 13.50) (7d, 10 mg), 13.1 ± 0.9 (12.38, 13.82) (7 days, 20 mg) vs. 11.2 ± 1.2 (10.24, 12.16); 14.3 ± 1.2 (13.34, 15.26) (11 days, 10 mg), 14.0 ± 1.09 (13.20, 14.80) (11 days, 20 mg) vs. 12.5 ± 1.1 (11.62, 13.38) 2. 53.1 ± 14.4 (41.58, 64.62) (7 days, 10 mg), 60.8 ± 17.4 (46.88, 74.72) (7 days, 20 mg) vs. 57.9 ± 17.3 (44.06, 71.74); 108.5 ± 29.6 (84.82, 132.18) (11 days, 10 mg), 88.5 ± 2.9 (86.18, 90.82) (11 days, 20 mg) vs. 96.6 ± 27.9 (74.28, 118.92); 40.9 ± 13.4 (30.18, 51.62) (21 days, 10 mg), 41.9 ± 12.7 (31.74, 52.06) (21 days, 20 mg) vs. 34.8 ± 11.9 (25.28, 44.32)	1.$P < 0.05$ (10, 20 mg/kg, 7, 11 days)
										BrdU-positive cells (10, 20 mg/kg; SGZ)		
												2.$P > 0.05$ (10, 20 mg/kg, 7, 11, 21 days)

Study (years)	Type of herbal or bioactive compound	Species (sex, n)	Weight, g	Anesthetic	Model	Ischemic time (min)	Induced method	Intervention: Treatment group	Intervention: Control group	Outcome measure	Treat group vs. model control group Mean ± SE (95% CI)	Intergroup differences
Yang et al, 2015[73]	L-3-n-butylphthalide	SD rat (male, 12/12)	—*	Chloral hydrate (400 mg/kg)	MCAO	120	Modified Longa 1989	30 mg/kg, i.g., once daily for 28 days	Vegetable oil	Escape latency (Morris)	1. NR	$P<0.05$
										BrdU-positive cells (DG)	2. NR	$P<0.01$
										BrdU/NeuN positive cells (DG)	3. NR	$P<0.01$
										BrdU/GFAP positive cells (DG)	4. NR	$P>0.05$

CA1 = cornu ammonis 1, CCAOAC = common carotid arteries occluded with aneurysm clips, DG = dentate gyrus, DMSO = dimethyl sulfoxide, i.g = intragastrical administration, i.p = intraperitoneal administration, LVI = lateral ventricle injection, MCAO = middle cerebral artery occlusion, NR = not reported, NSS = neurological severity score, SGZ = subgranular zone, SVZ = subventricular zone, VAIO = vertebral arteries were irreversibly occluded.
* Represents the lack of information.

8 studies,[47,55,56,62–64,66,67] halothane was used in 1 study,[46] and the remaining 4 studies did not report what kind of anesthetic was used.[59–61,68] Sixteen of 22 studies employed middle cerebral artery occlusion (MCAO) as the model of brain ischemia with the occlusion time varying from 1 to 2 hours,[44,45,48–53,55–58,61,65,72,73] whereas the remaining 6 studies utilized permanent MCAO model.[54,59,60,62–64] Seven studies were transient global ischemic models induced by using common carotid arteries occlusion plus irreversibly vertebral arteries occlusion or common carotid arteries occlusion plus hypotension.[46,47,66–70] Transient forebrain ischemic model was induced by using common carotid arteries occlusion in 1 study.[71] Common carotid arteries were completely blocked ranging from 6 to 30 minutes in the 8 studies. The treatment was administrated via intraperitoneal injection in 23 studies,[46–48,50,51,53–56,58–68,70–72] intragastric administration in 6 studies,[44,45,52,57,69,73] and lateral ventricle injection in 1 study.[49] The treatment effect was estimated by using 3 different kinds of neurogenesis outcome measures: 26 studies reported proliferation data as BrdU and/or Nestin markers;[44,45,50–73] 5 studies reported migration data as PSA-NCAM or DCX biomarker;[45,46,48,49,51] and 10 studies reported differentiation data as NeuN or GFAP biomarker.[45,47,48,54,56,57,59,67,70,73] Neurobehavioral assessment was reported in 16 studies.[44–46,48,49,52–55,57,66–69,72,73] Infarct volume was reported in 5 studies.[45,48–50,56] All experiments solely adopted certain kind of bioactive components of CHM in the treatment group and corresponding vehicle in the control group. Twenty-one bioactive components of CHM assessed their effects on neurogenesis after experimental ischemic stroke as follows: ligustrazine-treated in 4 studies,[61–64] ginsenoside Rgl-treated in 3 studies,[59,60,71] curcumin-treated in 2 studies,[50,51] salvianolic acid B used in 2 studies,[58,66] baicalin used in 2 studies,[67,69] L-3-n-butylphthalide used in 2 studies,[72,73] and bilobalide,[44] gypenosides,[45] resveratrol,[46] total saponins of Panax notoginseng,[47] ginsenoside Rd,[48] EGb 761,[49] soy lsoflavones,[52] quercetin,[53] ginseng total saponins,[54] ginsenoside Rb1,[55] tetramethylpyrazine,[56] cornel iridoid glycoside,[57] protoparaxotriol aponins,[65] puerarin,[68] and astragal side IV[70] used in each out of the remaining studies (Table 2).

3.3. Study quality

The score of study quality checklist items ranged from 2 of 10 to 7 of 10. Of whom, One study got 7 of 10 points,[48] 3 studies got 6 of 10 points,[46,47,55] 5 studies got 5 of 10 points,[49–51,57,67] 12 studies got 4 of 10 points,[44,45,53,54,58,63,64,66,69,70,72,73] 4 studies got 3 of 10 points,[52,56,62,65] and 5 studies got 2 of 10 points.[59–61,68,71] All the included studies were peer-reviewed and formally published, except 2 studies that were online master's theses.[69,72] Control of temperature as room temperature and rectal temperature of rats was described in 18 studies.[46–51,53,55,58–60,63,64,66,67,69,70,72] Random allocation was described in 22 studies.[44–55,57,58,61–65,69,70,72] Except 4 studies with no-report of anesthetic used,[59–61,68] all the included trials using anesthetics were without significantly intrinsic neuroprotective activity. Two studies declared outcome assessment with blindness.[44,48] None of the included studies described the blindness of model induction and a sample size calculation. An appropriate animal model that is relevant to the clinical situation such as aged animals, hyperglycemia, or hypertension was not used in all the studies. Sixteen studies reported compliance with animal welfare

Table 2

Bioactve compounds of included studies.

Bioactive compounds	Main biological source: Latin name	English name	Chinese name (Pinyin)	Effective dose	Model of neurological disease	Neurogenesis activity	Reference
Total saponins of Panax notoginseng	Radix Notoginseng	Sanchi	Sanqi	75 mg/kg	Ischemic brain injury	Increase cell migration and differentiation of olfactory bulb	He et al, 2015[47]
Resveratrol	Veratrum album	White hellebore	Baililu	1 mg/kg, 10 mg/kg	Ischemic brain injury	decrease cell migration of hippocampus	Girbovan et al, 2015[46]
Ginsenoside Rd	Radix Ginseng	Ginsenoside	Renshen	5 mg/kg	Ischemic brain injury	Increase cell proliferation, migration and differentiation of hippocampus	Liu et al, 2015[48]
Bilobalide	Folium Ginkgo	Ginkgo leaf	Yinxing	10 mg/kg	Ischemic brain injury	Increase cell proliferation in ipsilateral cortex	Zheng et al, 2013[44]
Gypenosides	Gynostemma pentaphyllum (Thunb.) Makino	Herba gynostemmatis pentaphylli	Jiaogulan	200 mg/kg, 400 mg/kg	Ischemic brain injury	Increase cell proliferation, migration and differentiation of hippocampus	Wang et al, 2013[45]
EGb 761	Folium Ginkgo	Ginkgo leaf	Yinxing	0.525 mg/kg	Ischemic brain injury	Increase cell proliferation and migration of hippocampus	Sun et al, 2013[49]
Curcumin	Rhizoma Curcumae Longae	Turmeric	Jianghuang	300 mg/kg	Ischemic brain injury	Promote cell proliferation and migration of SVZ	Liu et al, 2013[50]; Cheng et al, 2013[51]
Soy Isoflavones	Glycine Max	Soybean	Dadou	60 mg/kg	Ischemic brain injury	Promote cell proliferation in hippocampus	Long et al, 2011[52]
Quercetin	Flos Sophorae	Sophora flower bud	Huaimi	50 mg/kg	Ischemic brain injury	Promote cell proliferation of SVZ	Zhang et al, 2011[53]
Ginseng total saponins (GTS)	Radix Ginseng	Ginseng	Renshen	25 mg/kg	Ischemic brain injury	Increase cell proliferation and differentiation	Zheng et al, 2010[54]
Ginsenoside Rb1	Radix Ginseng	Ginseng	Renshen	40 mg/kg	Ischemic brain injury	Increase cell proliferation	Gao et al, 2010[55]
ginsenoside Rgl	Radix Ginseng	Ginseng	Renshen	5 mg/kg; 20 mg/kg;	Ischemic brain injury	Promote cell proliferation and differentiation in hippocampus and SVZ	Cui et al, 2007A[59]; Cui et al, 2007B[60]; Shen et al, 2003[71]
Tetramethylpyrazine	Rhizoma Ligustici Chuanxiong	Szechwan lovage rhizome	Chuanxiong	20 mg/kg	Ischemic brain injury	Increase cell proliferation and differentiation	Xiao et al, 2010[56]
Cornel iridoid glycoside	Fructus Corni	Asiatic cornelian cherry fruit	Shanzhuyu	60 mg/kg; 180 mg/kg	Ischemic brain injury	Increase cell proliferation and differentiation	Yao et al, 2008[57]
Salvianolic acid B	Radix Salviae Miltiorrhizae	Danshen root	Danshen	10 mg/kg; 50 mg/kg	Ischemic brain injury	Increase cell proliferation in SVZ and SGZ	Zhong et al, 2007[58]; Zhuang et al, 2012[66]
Ligustrazine	Rhizoma Ligustici Chuanxiong	Szechwan lovage rhizome	Chuanxiong	40 mg/kg; 80 mg/kg	Ischemic brain injury	Increase cell proliferation in SVZ and SGZ	Qi et al, 2006[61]; Qiu et al, 2006A[62]; Qiu et al, 2006B[63]; Qiu et al, 2006C[64]
Protoparaxotriol aponins	Radix Notoginseng	Sanchi	Sanqi	50 mg/kg	Ischemic brain injury	Increase cell proliferation in border area of infarct	Hu et al, 2004[65]
Puerarin	Radix Puerariae	Kudzuvine root	Gegen	—	Ischemic brain injury	Increase cell proliferation in SVZ	Li et al, 2012[68]
Astragaloside IV	Radix Astragali seu Hedysari	Milkvetch root	Huangqi	2 mg/kg; 4 mg/kg	Ischemic brain injury	Increase cell proliferation and differentiation in hippocampus	Wang et al, 2009[70]
Baicalin	Radix Astragali seu Hedysari	Milkvetch root	Huangqi	50 mg/kg; 100 mg/kg	Ischemic brain injury	Increase cell proliferation and differentiation in SVZ and SGZ	Li, 2011[69]; Zhuang et al, 2013[67]
L-3-n-butylphthalide	—	Celery seed	Dingbentai	10 mg/kg; 20 mg/kg;30 mg/kg	Ischemic brain injury	Increase cell proliferation and differentiation in SGZ and DG zone	Hou, 2007[72]; Yang et al, 2015[73]

SGZ = subgranular zone, SVZ = subventricular zone, VAIO = vertebral arteries were irreversibly occluded.

Table 3

Risk of bias of included studies.

Study	A	B	C	D	E	F	G	H	I	J	Total
Zheng et al, 2013[44]	+	−	+	−	+	+	−	−	−	−	4
Wang et al, 2013[45]	+	−	+	−	−	+	−	−	+	−	4
Girbovan et al, 2015[46]	+	+	+	−	−	+	−	−	+	+	6
He et al, 2015[47]	+	+	+	−	−	+	−	−	+	+	6
Liu et al, 2015[48]	+	+	+	−	+	+	−	−	+	+	7
Sun et al. 2013[49]	+	+	+	−	−	+	−	−	+	−	5
Liu et al, 2013[50]	+	+	+	−	−	+	−	−	+	−	5
Cheng et al, 2013[51]	+	+	+	−	−	+	−	−	+	−	5
Long et al, 2011[52]	+	−	+	−	−	+	−	−	−	−	3
Zhang et al, 2011[53]	+	+	+	−	−	+	−	−	−	−	4
Zheng et al, 2010[54]	+	−	+	−	−	+	−	−	+	−	4
Gao et al, 2010[55]	+	+	+	−	−	+	−	−	+	+	6
Xiao et al, 2010[56]	+	−	−	−	−	+	−	−	+	−	3
Yao et al, 2008[57]	+	−	+	−	−	+	−	−	+	+	5
Zhong et al, 2007[58]	+	+	+	−	−	+	−	−	−	−	4
Cui et al, 2007A[59]	+	+	−	−	−	?	−	−	−	−	2
Cui et al, 2007B[60]	+	+	−	−	−	?	−	−	−	−	2
Qi et al, 2006[61]	+	−	+	−	−	?	−	−	−	−	2
Qiu et al, 2006A[62]	+	−	+	−	−	+	−	−	−	−	3
Qiu et al, 2006B[63]	+	+	+	−	−	+	−	−	−	−	4
Qiu et al, 2006C[64]	+	+	+	−	−	+	−	−	−	−	4
Hu et al, 2004[65]	+	−	+	−	−	+	−	−	−	−	3
Zhuang et al, 2012[66]	+	+	−	−	−	+	−	−	+	−	4
Zhuang et al, 2012[67]	+	+	−	−	−	+	−	−	+	+	5
Li et al, 2012[68]	+	−	−	−	−	+	−	−	−	−	2
Li, 2011[69]	−	+	+	−	−	+	−	−	+	−	4
Wang et al, 2009[70]	+	+	+	−	−	+	−	−	−	−	4
Shen et al, 2003[71]	+	−	−	−	−	+	−	−	−	−	2
Hou, 2007[72]	−	+	+	−	−	+	−	−	+	−	4
Yang et al. 2015[73]	+	−	−	−	−	+	−	−	+	+	4

Studies fulfilling the criteria of: A, peer reviewed publication; B, control of temperature; C, random allocation to treatment or control; D, blinded induction of model; E, blinded assessment of outcome; F, use of anesthetic without significant intrinsic neuroprotective activity; G, animal model (aged, diabetic, or hypertensive); H, sample size calculation; I, compliance with animal welfare regulations; J, statement of potential conflict of interests. + = Yes, − = No, ? = unclear.

regulations.[45–51,54–57,66,67,69,72,73] Seven studies manifested no potential conflicts of interest.[46–48,55,57,67,73] The methodological quality of each study was summarized in Table 3.

3.4. Effectiveness

3.4.1. Neurobehavioral assessment. Sixteen studies reported neurobehavioral assessment.[44–46,48,49,52–55,57,66–69,72,73] Neurological deficits' score was assessed in 10 studies by using either Longa criterion, Bederson criterion, or neurological severity score, which indicated that bioactive components of CHM including bilobalide,[44] gypenosides,[45] ginsenoside Rd,[48] EGb 761,[49] soy lsoflavones,[52] quercetin,[53] ginseng total saponins,[54] ginsenoside Rb1,[55] cornel iridoid glycoside,[57] and butylphthalide[72] showed significant lower neurological deficiency ($P<0.01$ or $P<0.05$). The remaining 6 studies performing Morris water-maze test showed that resveratrol,[44] salvianolic acid B,[66] baicalin,[67,69] puerarin,[68] and butylphthalide[73] significantly decreased escape latency and increased residence time in the target quadrant ($P<0.05$).

3.4.2. Infarct volume. Of the 5 studies reporting infarct volume, the bioactive components of CHM including gypenosides,[45] ginsenoside Rd,[48] EGb 761,[49] curcumin,[50] and tetramethylpyrazine[56] in treatment groups significantly reduced infarct volume when compared with the corresponding control groups ($P<0.05$).

3.4.3. Neurogenesis outcomes. Twenty-six of all the included studies reporting proliferation data as BrdU and/or Nestin showed that bilobalide,[44] gypenosides,[45] curcumin,[50,51] soy lsoflavones,[52] quercetin,[53] ginseng total saponins,[54] ginsenoside Rb1,[55] tetramethylpyrazine,[56] cornel iridoid glycoside,[57] salvianolic acid B,[58,66] ginsenoside Rgl,[59,60,71] ligustrazine treated in 4 studies,[61–64] protoparaxotriol aponins,[65] baicalin,[67,69] puerarin,[68] astragaloside IV,[70] and L-3-n-butylphthalide[73] significantly increased the expression of BrdU and/or Nestin in rats/mice brain after ischemic injury ($P<0.05$, or $P<0.01$), with exception in 1 study ($P>0.05$).[72]

Of the 5 studies separately adopting PSA-NCAM and DCX markers to assess the migration of NSCs, 4 bioactive components of CHM including gypenosides,[45] ginsenoside Rd,[48] EGb 761,[49] and curcumin[51] significantly promoted the expression of PSA-NCAM and DCX in dentate gyrus, SVZ, or olfactory bulb ($P<0.05$). The remaining 1 study reported that resveratrol significantly attenuated the expression of DCX/PSA-NCAM in DG region ($P<0.05$).[46]

Of the 10 studies reporting differentiation data as NeuN or GFAP, 5 studies demonstrated that bioactive components of CHM including total saponins of panax notoginseng,[47] ginseng total saponins,[54] tetramethylpyrazine,[56] cornel iridoid glycoside,[57] and baicalin[67] had significant effect on improving the expression of NeuN in ipsilateral infarct area, SGZ, or olfactory bulb ($P<0.05$); 7 studies reported that bioactive components of CHM including gypenosides,[45] ginsenoside Rd,[48] tetrame-

thylpyrazine,[56] cornel iridoid glycoside,[57] ginsenoside Rgl,[59] and astragaloside IV[70] had significant effect on promoting the expression of GFAP in SVZ, striatum, ipsilateral infarct area, or SGZ region ($P<0.05$), whereas ginseng total saponins showed no significant effect on GFAP expression after ischemic stroke ($P>0.05$).[54]

4. Discussion

4.1. Principle finding of the study

To our knowledge, this is the first preclinical systematic review evaluated the efficacy of bioactive components of CHM for neurogenesis. The present study showed that bioactive components of CHM can improve neurological dysfunction, reduce infarct volume, and promote endogenous neurogenesis, including proliferation, migration, and differentiation of NSCs after ischemic stroke.

4.2. Limitations

In the present study, some limitations have been identified. First, in spite of systematic search strategy, other language studies have not been taken into consideration except English and Chinese studies, which may lead to certain degree of selective bias.[74] Furthermore, except the 3 studies of Hou,[72] Girbovan et al,[46] and Zheng et al[54] in respect of proliferation, migration, and differentiation outcome, respectively, all the included studies concluded positive results. Some negative studies missed inevitability, as authors or researchers were unlikely to put effort in publishing negative results and positive ones would be more acceptable in publishing. Thus, the overall effect in this review may be overestimated. Second, study quality was considered as low, which indicated that the results should be explained with caution. The quality of the included studies was a significant predictor of outcome. The dominance of positive studies might imply presence of flaws in randomization and blinding.[75] Third, as high heterogeneities were inherent in the included studies, meta-analyses of the outcome measures can hardly be performed, which effectively pools into single quantitative estimate and summary effect size based on statistical techniques, otherwise.

4.3. Implication for further studies

The most critical step in the fundamental recovery of brain function was reconstruction of neuronal networks, including neuritic regeneration and synaptic reconstruction.[76] None of the included studies investigated whether the newborn neurons integrated into neuronal networks with functional properties. Therefore, further research should pay close attention to the newborns physiological function by electrophysiology and other methodologies.

In the present study, none of the included studies reported the blindness of model induction and a sample size calculation. Randomization was declared in most of the included studies, whereas none of them reported details of how the animals were randomized. Landis et al[77] suggested the core standards of rigorous study design including randomization, blinding, sample-size estimation, and the handling of all data should be depicted in detail. None of models were established on aged, diabetic, or hypertensive animals. The relevance of animal models with normal physiological conditions to human conditions remains dubious.[78] We suggest that the ARRIVE[79] should be used as a guideline when designing and reporting preclinical animal studies.

In conclusion, bioactive components of CHM may improve neurological dysfunction, reduce infarct volume, and promote endogenous neurogenesis, including proliferation, migration, and differentiation of NSCs after ischemic stroke. However, evidences are supported but limited because only a few studies were available for each descriptive analysis. Further research is needed to update supporting the evidence in this area.

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