A Systematic Review on Neuroprotective Effects of Catalpolin Acute Focal Ischemic Stroke and Their Possible Mechanisms

ABSTRACT

This study aims to systematically review the evidence on the neuroprotective effects of Catalpol in animal models of acute focal ischemic stroke and to determine its potential mechanisms of action.Catalpolis the primary active ingredient extracted from the roots of Radix Rehmanniae. Catalpol is found to have pleiotropic neuroprotective effects in neurodegenerative conditions like ischemic stroke. In this study, neuroprotective effects of Catalpolare explored in experimental acute ischemic stroke.

Studies relevant to effects of Catalpol in animal models of acute ischemic stroke were identified from the following databases ,PubMed, Wanfang Data information site, Chinese National Knowledge Infrastructure (CNKI), and VIP information database and the time period for the study search was fixed as 2010 to 2022.Methodological quality of the included studies was assessed using the CAMARADES checklist. As per the inclusion criteria, twenty-one studies were included.Six out of eightstudies had shown significant impact of Catalpol in reducing infarct size according to 2,3,5-triphenyltetrazolium chloride staining compared with the control ($P < 0.05$). And two studies that determined infarct size by magnetic resonance imaging did not report any significant effect of Catalpol in infarct size reduction when compared to the control.

Several tests were used in the studies to assess neurological function score of the animal models, such as Zea Longa score, Balance beam-walking test, Bederson score, adhesive removal test, muscular strength test, bar-grasping test, corner test, cylinder test, neuromuscular function test and foot-fault test. However, ladder rung walking test, sensorimotor function test, open-field test, horizontal ladder test and novel object recognition test were used each in one of the studies.

To conclude, Catalpol exerts neuroprotective effects in animal models of acute focal ischemic stroke, mainly by alleviating oxidative stress, inhibiting apoptosis, and repressing inflammatory reactions and autophagy. However, due to the methodological flaws, such as risk of publication bias due to predominance of Chinese studies and low methodological quality of included studies, these positive findings should be interpreted and analyzed with caution.

Keywords:*Catalpol,Acute ischemic stroke, Radix Rehmanniae, Infarct Volume, Neurological function score*

1. INTRODUCTION

The term "neuroprotection" describes the idea of treating brain tissue in the ischemic penumbra while it is still viable in order to prevent or lessen the effects of an infarction [1,2]. “Preventing local inflammation, excitotoxicity, free radical damage, neuronal apoptosis,

and calcium influx into cells are potential mechanisms of neuroprotective treatments, which can improve functional outcomes and reduce infarct size" [3].

"Research on developing various neuroprotective therapies that can lessen brain damage after ischemic stroke in animal models has been abundant in the last few decades" [4]. "Unfortunately, effective neuroprotective treatments for stroke patients have not yet been found in clinical trials. It is thus critically necessary to find or develop novel neuroprotectants, given the enormous translational gap between these animal studies and clinical trials" [5]."The first mention of the roots of Radix RehmanniaeRecens, known by the Latin name Radix Rehmanniae, the English name Rehmannia root, and the Chinese name Dihuang, can be found in the book Shennongbencaojing, also known as Shennong's Classic of Materia Medica, which is the earliest comprehensive pharmacopoeia in China. In China and other parts of the world, prescriptions containing Radix Rehmanniae and its derivatives are still commonly used today to treat a variety of illnesses" [6, 7]. Among other systems, the endocrine, blood, immune, neurological, cardiovascular, and so on are all affected pharmacologically by Radix Rehmanniae [8].

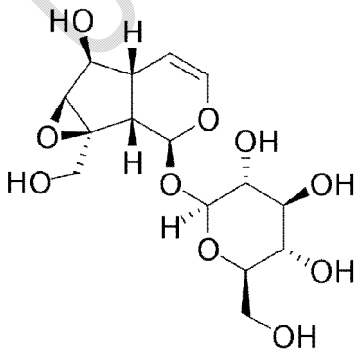
Catalpol, the primary active compound found in the root of Radix Rehmanniae, has been shown in recent studies to have diverse neuroprotective effects against various conditions such as hypoxic/ischemic injury, Alzheimer's disease, and Parkinson's disease in both laboratory and animal models. These studies have demonstrated that Catalpol possesses antioxidative, anti-inflammatory, anti-apoptotic, and other neuroprotective properties, indicating its potential as a treatment for stroke. [9, 10]

"Systematic reviews are considered the highest level of medical evidence, with only data from systematic reviews categorized as 1a evidence according to the levels of evidence from the Centre of Evidence-Based Medicine in Oxford" [11]. "A new approach known as preclinical systematic reviews has been developed to evaluate and synthesize results from animal research into a single document, guiding further basic research, refining experimental studies, and increasing success rates in future clinical trials".[12] To date, no systematic analysis has been conducted on the efficacy of Catalpol for experimental ischemic stroke. Therefore, our objective is to review the current evidence on Catalpol as a neuroprotective agent in animal models of acute focal ischemic stroke.

1.1 General Information on Catalpol

Catalpol was first separated from the plants of Genus Catalpa in 1962. Later it was known that some plants of genus Rehmannia contains large amounts of Catalpol in 1969. Later, Catalpol was also found to be present in various plant families of Lamiales such as Scrophuriaceae, Lamiaceae, Plantaginaceae and Biogoniaceae. [13] [14]

Figure 1: Structure of Catalpol



Catalpol is an iridoid glucoside extracted from the plant roots of Rehmanniaglutinosa. It can also be obtained from Semen plantaginis and Fructus catalpa. Based on nuclear magnetic resonance spectra, along with other physical and chemical evidences, the structure for Catalpol was developed, which is as follows, [14]

The molecular formula of Catalpol is C15H22O10 and the molecular weight is 362.45. The melting point of Catalpol ranges between 207 – 209 degrees Celsius. Catalpol is an optically active substance, which is colourless, amorphous and hygroscopic. Catalpol is soluble in water, ethanol and methanol while not easily soluble in organic solvents like chloroform, benzene, petroleum ether having high lipophilicity. [15] Catalpol is acid-unstable as its glycoside bonds and is also easily hydrolysable. Catalpol degrades at 100 degrees Celsius and is unstable at higher temperatures. [13]

2. METHODOLOGY

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist as a guide, we conducted this report. Registration number for this study with PROSPERO is CRD42023337830

2.1 Search Strategy

The following four electronic databases were searched independently by two authors: PubMed, Wanfang Data information site, Chinese National Knowledge Infrastructure (CNKI), and VIP information database. They looked for pertinent experimental studies of Catalpol for acute focal ischemic stroke. Throughout the systematic search process, disagreements were discussed and settled with the third author. Chinese databases were searched using the following search terms: "Catalpol" AND ["ischemic stroke" OR "cerebral infarct" OR "middle carotid artery occlusion (MCAO)" OR "cerebral ischemia/reperfusion"]. All animal studies published from the databases' creation to September 2023 were searched without regard to language restrictions. "Catalpol" was the term that was used in English databases.

2.2 Study Selection

2.2.1 Inclusion Criteria

1) Participants: acute permanent MCAO or temporary MCAO animal models, irrespective of differences in species, sex, and modeling method;
2) Intervention: Catalpol, variations allowed in dosage, timing, and frequency of administration
3) Comparison: Either non-functional substance like water or normal saline, or no treatment at all, at the same dose and mode of administration
4) Outcomes: the primary outcome being measured will be neurological function score (NFS) and/or infarct volume (IV)

2.2.2 Exclusion Criteria

1) The study was not an animal study
2) The study was an *in vitro*, in-humans, or *in silico*
3) It was a review, case report, commentary, abstract, or editorial
4) It was not a study on acute focal cerebral ischemia model, such as traumatic, global, chronic cerebral ischemic models or not cerebral ischemic models;
5) The study had an animal model of non-acute focal ischemic stroke

6) Catalpol was not used as a monotherapy;
7) neither NFS nor IV was used as one of the outcome measurements;
8) there was no control group in the study;
9) the article was a duplicate publication.

2.3 Quality Assessment

Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) 10-item checklist was used to assess the methodological quality of each included study.[16] The items on the checklist were: (1) peer-reviewed publication; (2) statements describing temperature control; (3) randomization of animals to treatment group; (4) allocation concealment; (5) blinded outcome assessment; (6) avoidance of anesthetics with known notable intrinsic neuroprotective properties; (7) use of animals with relevant comorbidities; (8) sample size calculation; (9) compliance with animal welfare regulations; and (10) declaration of any potential conflict of interest. Every item on this scale was given one point in order to calculate the overall quality score. ZXW and YWT, two writers, separately gathered data and assessed the caliber of the research. A discussion of the study's specifics resolved disagreements.

2.4 Data extraction

After deduplication, two authors independently performed literature screening based on the inclusion and exclusion criteria. First, included studies were initially screened by reading their titles and abstracts to exclude irrelevant articles; the full texts of the studies were then systematically reviewed for their inclusion in the review. The following information of each included study was extracted:

Using the inclusion and exclusion criteria, two authors independently screened the literature after deduplication. The full texts of the included studies were then methodically examined for inclusion in the review after the included studies were first filtered by examining their titles and abstracts to weed out irrelevant articles.

1) the first author's name and year of publication

2) study animal’s sex, species, weight, and animal number;

3) information about the animal model, including ischemic time, permanent or temporary MCAO, the anesthetic used, and the random method

4) treatment information, including the drug used, treatment method, timing of the initial treatment, and treatment duration;

5) Measures of outcomes, timing for outcome evaluations, and between-group differences. NFS and/or IV was extracted independently. We extracted information from the most recent time point if results were shown at different times. We extracted data from the highest dose of Catalpol if studies used a drug's dose gradient because of the dose-response relationship and prespecific criteria. The third author was consulted during the data extraction process to clarify any ambiguous findings. We attempted contacting the authors to obtain the necessary data or to calculate it using the appropriate software if the data were lacking or displayed in graphs.

The following details were taken from Rehmanniae Radix's mechanism studies of Catalpol for experimental ischemic stroke: the first author's name, the year of publication, the models

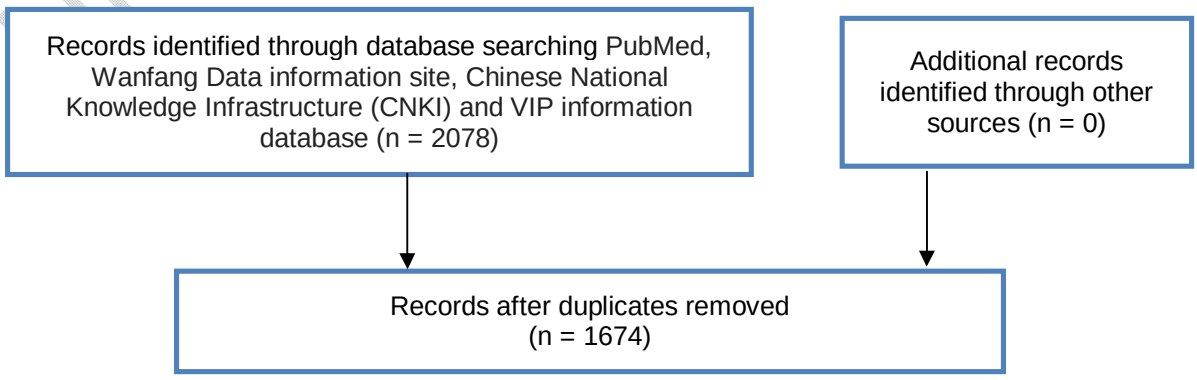
used in the experiment, the interventions in the experimental and control groups, the observation, and potential mechanisms.

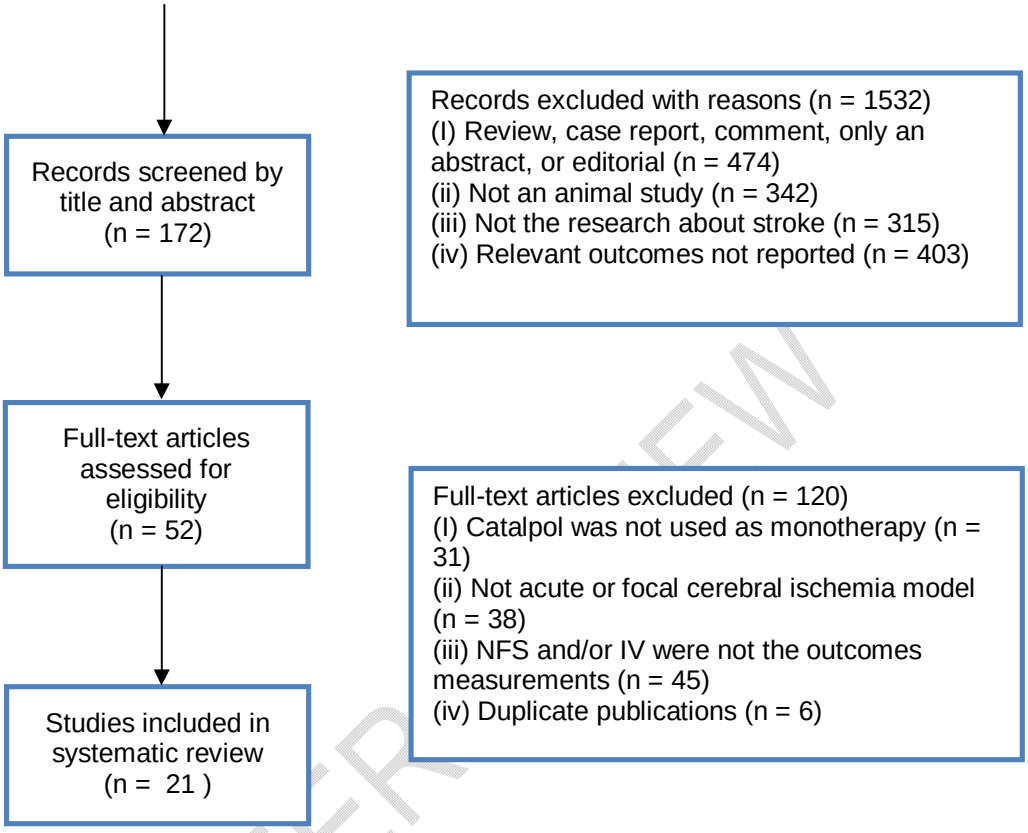
3. RESULTS

3.1 Study Selection

We conducted systematic searches across four databases and found 2078 papers. 1674 records were left after 404 duplicate articles were eliminated.The following criteria were used to isolate 172 articles from the 1674 articles based on their titles and abstracts, thus excluding 1502 articles: (1) the article was a review, case report, comment, abstract, or editorial; (2) it was not an animal study; (3) it was not a study on cerebral ischemia or stroke; and (4) it did not report pertinent outcomes. Out of the resultant 172 articles isolated, 31 studies were eliminated because Catalpol was not used as a monotherapy; 38 studies were eliminated because acute focal cerebral ischemia was not the animal model; 45 studies were eliminated because the outcome measurement was neither IV nor NFS; and six studies were eliminated because they were duplicate publications. 21 articles were ultimately found to be eligible.

Figure 2- Flow chart of the systematic search





3.2 Risk of Bias and Quality of Included Studies

The quality scores of the 21 included studies ranged from 2 to 7 points. One study [24] got 2 points; 6 studies [18, 20, 26, 28, 30, 31] got 3 points; 10 studies [19, 20, 21, 23, 25, 27, 33, 34, 35, 37] got 4 points; 2 studies [32, 35] got 5 points; one study [17] got 6 points; one study [29] got 7 points (Table 1). The average score was 3.952. Eight studies described control of temperature [18, 21, 25, 27, 29, 32, 35, 36]. Random allocation to treatment group was described in 14 studies [17, 19, 20, 22, 23, 24, 25, 26, 27, 29, 32, 33, 34, 35]. No study reported allocation concealment. Blinded assessment of outcome was described in 4 studies [17, 29, 36]. “Nineteen studies did not use anesthetics with significant intrinsic neuroprotective activity, and the remaining 2 studies did not report the type of anesthetics” [18, 24]. “No study used animals with relevant comorbidities. No study described the sample size calculation. Fourteen studies reported compliance with animal welfare regulations” [17-19, 21-23, 29-34, 36, 37]. Twelve studies mentioned statement of potential conflict of interests [17, 18-24, 28, 29, 34, 36, 37].

Table 1: Risk of Bias

S. No	Study (years)	1	2	3	4	5	6	7	8	9	10	Total
17)	Zhu et al. (2010)	+	-	+	-	+	+	-	-	+	+	6
18)	Wan et al. (2013)	+	+	-	-	-	NR	-	-	+	-	3
19)	Wang Hongli at al. (2013)	-	-	+	-	-	+	-	-	+	+	4

20)	Xue Lijun et al. (2012)	-	-	+	-	-	+	-	-	-	+	3
21)	Wang Tao et al. (2015)	-	+	-	-	-	+	-	-	+	+	4
22)	Qin Lie et al. (2016)	-	-	+	-	-	+	-	-	+	+	4
23)	Zhang Fen et al. (2011)	-	-	+	-	-	+	-	-	+	+	4
24)	Liu Ming et al. (2011)	-	-	+	-	-	NR	-	-	-	+	2
25)	Zhang Shenwei et al. (2013)	+	+	+	-	-	+	-	-	-	-	4
26)	Tan Lingli et al. (2014)	+	-	+	-	-	+	-	-	-	-	3
27)	Liu at al. (2011)	+	+	+	-	-	+	-	-	-	-	4
28)	Zhang Xiaushang et al. (2016)	+	-	-	-	-	+	-	-	-	+	3
29)	Wan Dong et al. (2016)	+	+	+	-	+	+	-	-	+	+	7
30)	Zhiming et al. (2017)	+	-	-	-	-	+	-	-	+	-	3
31)	Jin Wang et al. (2021)	+	-	-	-	-	+	-	-	+	-	3
32)	Jinghui Wang et al. (2022)	+	+	+	-	-	+	-	-	+	-	5
33)	Hui Feng Zhu et al. (2019)	+	-	+	-	-	+	-	-	+	-	4
34)	Shao Yali et al. (2020)	-	-	+	-	-	+	-	-	+	+	4
35)	Huang Tao et al. (2019)	+	+	+	-	-	+	-	-	-	-	4
36)	Wantong at al, 2012	-	+	-	-	+	+	-	-	+	+	5
37)	Wantong et al, 2013	+	-	-	-		+	-	-	+	+	4

3.3 Study Characteristics

21 studies with 1135 animals were included. Among them, 6 studies were published in English and 15 studies were Chinese papers between 2010 and 2022. 18 studies used male and/or female Sprague Dawley (SD) rats and three studies [21, 19, 20] used Kunming mice. The weight of SD rats used varied from 180 g to 350 g; the weight of mice varied from 22 g to 30 g. Chloral hydrate was used to induce anesthesia in 11 studies and isoflurane in one study [31]; while the remaining 9 studies did not report the type of anesthetics [18, 24, 30, 32, 33, 34, 35, 36, 37]. Eighteen out of the 21 studies utilized permanent MCAO models, and the remaining three studies [21, 24, 36] were temporary MCAO models in which ischemic time varied from 1 to 2 hours.

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Thirteen studies utilized a dose gradient of Catalpol: four studies [24, 25, 27, 28] administrated 15, 30, and 60 mg kg^{-1} intragastrically, four studies [19, 21, 23, 36] used 1, 5, and 10 mg kg^{-1} intraperitoneally, one study [31] used 2.5, 5.0, 10 mg kg^{-1} intraperitoneally, three studies [22, 33, 34] used 5 and 10 mg kg^{-1} intraperitoneally, one study [19] used 1.42, 7, and 14.2 mg kg^{-1} intraperitoneally, and one study [35] used 10, 20 mg kg^{-1} intraperitoneally. While the remaining eight studies used fixed dose of Catalpol; four studies [17, 26, 29, 37] used 5 mg kg^{-1} intraperitoneally, two studies [18, 20] used 9 mg kg^{-1} intraperitoneally, one study [30] administered 15 mg kg^{-1} by gavage, and one study [32] used 10 mg kg^{-1} intraperitoneally.

Nineteen studies administrated Catalpol after stroke and two studies administrated Catalpol before and after stroke [19, 21]. In the control group, 13 studies used normal saline solution in the control group; 1 study [24] applied edible oil; 2 studies [27, 30] applied distilled water; one study [23] had used 1,2-propylene glycol; 2 studies [25, 28] applied edible oil and normal saline; and 2 studies [29, 32] used no treatment.

Ten studies [18, 19, 20, 21, 23, 25, 31, 32, 36, 37] adopted IV as outcome measurements; twenty-one studies used NFS as outcome measurements; and 10 studies adopted both above two outcome measurements. However, different methods that were used to identify IV; 8 studies used TTC staining and 2 studies [36, 37] used MRI scan.

The standards of NFS were diverse: 7 studies [17, 20, 23, 24, 25, 27, 28] adopted Zea Longa (ZL) score; 7 studies [17, 19, 23, 24, 25, 26, 27] used balance beam-walking test; 5 studies [17, 19, 21, 22, 34] used Bederson score; 4 studies [24, 25, 27, 37] used adhesive removal test; 2 studies [19, 34] used muscular strength test; 2 studies [24, 25] used bar-grasping test; 3 studies [21, 22, 36] used corner test; and 2 studies [26, 29] used cylinder test. From another perspective, neuromuscular function test [19, 21] and foot-fault test [29, 37] were used to determine NFS in 2 studies. Ladder rung walking test [22], sensorimotor function test [37], open-field test [30], horizontal ladder test [33], novel object recognition test [30] was utilized in 1 study. The basic characteristics of the study are shown in the table given below;

Table 2: Study Characteristics

S.no	Study (years)	Species(sex,exp/control group),weight	Model / Method	Anesth-eticUsed	Method of administration		Outcome index (time)	Inter-group differences
					Experimental group	Control group		
17.	Zhu et al., 2010	SD rats (male, 24/24), 220–280 g	Permanent MCAO	Chloral hydrate	Catalpol (5 mg kg^{-1}, intraperitoneally (ip)); 24 h after occlusion; then daily for 7 d	Normal saline (same volume, ip); 24 h after occlusion; then daily for 7 d	(1) NFS (Bederson score, 1, 4, 7, and 15 d) (2) NFS (balance beam-walking test, 1, 4, 7, and 15 d) (3) VWF and PCNA	(1) $P < 0.05$ (2) $P < 0.05$ (3) $P < 0.05$ (4) $P < 0.05$ (5) $P < 0.05$ (6) $P < 0.05$ (7) $P < 0.05$

							colocalization point count (4) EPO expression (5) VEGF expression (6) EPO-positive cell (7) VEGF-positive cell (8) Vascular pattern (9) Brain capillary endothelial cell microstructure	(8) NR (9) NR
18.	Wan et al. 2013	Kunming mice (both, 10/10), 25-30 g	Permanent MCAO	NR	Catalpol (9 mg kg^{-1}, ip); 24 h after occlusion; once daily for 3 d	Normal saline (same volume, ip); 24 h after occlusion; once daily for 3 d	(1) NFS (ZL score, 1, 2, and 3 d) (2) IV (TTC, 3 d) (3) Cerebral blood flow ratio	(1) $P < 0.01$ (2) $P < 0.05$ (3) $P < 0.05$
19.	Wang Hongli, 2013	Kunming mice (NR, 6/6), 23 – 28 g	Permanent MCAO	3.5% chloral hydrate (10 mL kg^{-1})	Catalpol (1.42, 7, and 14.2 mg kg^{-1}, ip); after occlusion; once daily for 3 d	Normal saline (same volume, ip); after occlusion; once daily for 3 d	(1) NFS (neuromuscular function test, 3 d) (2) NFS (muscular strength test, 3 d) (3) IV (TTC, 3 d)	(1) $P > 0.05$ (2) $P > 0.05$ (3) $P < 0.05$
		Kunming mice (NR, 6/6), 23 – 28 g	Permanent MCAO	3.5% chloral hydrate (10 mL kg^{-1})	Catalpol (14.2 mg kg^{-1}, ip); 0.5 h before, 1 h after or 24 h after occlusion; once daily for 3 d	Normal saline (same volume, ip); after occlusion; once daily for 3 d	(1) NFS (neuromuscular function test, 3 d) (2) NFS (muscular strength test, 3 d) (3) IV (TTC, 3 d)	(1) $P > 0.05$ (2) $P > 0.05$ (3) $P > 0.05$
		SD rats (NR, 8/8), 250 – 300 g	Permanent MCAO	3.5% chloral hydrate (10 mL kg^{-1})	Catalpol (1, 5, and 10 mg kg^{-1}, ip); after occlusion; once daily for 7 d	Normal saline (same volume, ip); after occlusion; once daily for 7 d	(1) NFS (Bederson score, 1, 4, 7, 15, 21, and 28 d) (2) NFS (muscular strength test, 1, 4, 7, 15, 21, and 28 d) (3) NFS (balance beam-walking test, 1, 4, 7, 15, 21, 28 d) (4) IV (TTC, 3 d)	(1) $P > 0.05$ (2) $P > 0.05$ (3) $P > 0.05$ (4) $P > 0.05$
20.	Xue Lijun, 2012	Kunming mice (both, 10/10), 22 – 28 g	Permanent MCAO	Chloral hydrate	Catalpol (9 mg kg^{-1}, iv); 24 h after occlusion; once daily for 3 d	Normal saline (same volume, iv); 24 h after occlusion; once daily for 3 d	(1) NFS (ZL score, 1, 2, and 3 d) (2) IV (TTC, 3 d) (3) Cerebral blood flow ratio (4) Hippocampal tissue morphology	(1) $P < 0.01$ (2) $P < 0.01$ (3) $P < 0.01$ (4) NR
21.	Wang Tao, 2015	SD rats (NR, 8/8), 250 – 350 g	Temporary MCAO/2 h	3.5% chloral hydrate (10 mL k	Catalpol (1, 5, and 10 mg kg^{-1}, ip); 12 h before and 1 h after	Normal saline (same volume, ip); 12 h before and 1 h after	(1) NFS (Bederson score, 1 d) (2) NFS (neuromuscular	(1) $P > 0.05$ (2) $P < 0.01$ (3) $P < 0.05$ (4) $P < 0.05$

				g^{-1})	occlusion	occlusion	function test, 1 d) (3) NFS (corner test, 1 d) (4) IV (TTC, 1 d) (5) LC3 express	(5) NR
22.	Qin Lei, 2016	SD rats (male, 6/6), 220–280 g	Permanent MCAO	3.5% chloral hydrate	Catalpol (5 and 10 mg kg^{-1}, ip); 6 h after occlusion; once daily for 21 d	Normal saline (100 g/1 mL, ip); 6 h after occlusion; once daily for 21 d	(1) NFS (Bederson score, 1, 3, 7, 14, and 21 d) (2) NFS (corner test, 1, 3, 7, 14, and 21 d) (3) NFS (ladder rung walking test, 1, 3, 7, 14, and 21 d)	(1) $P > 0.05$ (2) $P > 0.05$ (3) $P < 0.01$ (4) $P > 0.05$
23.	Zhang Fen, 2011	SD rats (male, 10/10), 180–220 g	Permanent MCAO	Chloral hydrate	Catalpol (NR, iv); 3 h after occlusion; once daily for 7 d	1, 2-Propylene glycol (NR, iv); 3 h after occlusion; once daily for7 d	(1) NFS (ZL score, 7 d) (2) NFS (balance beam-walking test score, 7 d) (3) IV (TTC, 7 d)	(1) $P < 0.01$ (2) $P < 0.01$ (3) $P < 0.05$
24.	Liu Ming, 2011	SD rats (male, 11/10), 260-280 g	Temporary MCAO/2 h	NR	Catalpol (15, 30, and 60 mg kg^{-1}, intragastrically (ig)); 2 d after occlusion; once daily for 12 d	Edible oil (same volume, ig); 2 d after occlusion; once daily for 12 d	(1) NFS (ZL score, 3, 7, 10, and 14 d) (2) NFS (balance beam-walking test, 3, 7, 10, and 14 d) (3) NFS (adhesive removal test, 3, 7, 10, and 14 d) (4) NFS (bar-grasping test, 3, 7, 10, and 14 d) .(5) Lactic acid content (6) Pyruvic acid content (7) Lactic acid content/pyruvic acid content (8) Na^+, K^+-ATPase activity (9) Ca^{2+}, Mg^{2+}-ATPase activity	(1) $P < 0.01$ (2) $P < 0.05$ (3) $P < 0.05$ (4) $P < 0.05$ (5) $P < 0.05$ (6) $P < 0.01$ (7) $P < 0.01$ (8) $P < 0.01$ (9) $P < 0.01$
25.	Zhang Shenwei, 2013	SD rats (male, 13/13), 210 – 240 g	Permanent MCAO	10% chloral hydrate (350 mg kg^{-1})	Catalpol (15, 30, and 60 mg kg^{-1}, ig); after occlusion	Normal saline (same volume, ig); after occlusion	(1) NFS (ZL score, 6 and 24 h) (2) IV (TTC, 24 h) (3) Brain water content (4) Water content	(1) $P <$ 0.001 (2) $P > 0.05$ (3) $P > 0.05$ (4) $P < 0.05$
		SD rats (male, 10/10), 260 – 290 g	Permanent MCAO	10% chloral hydrate	Catalpol (15, 30, and 60 mg kg^{-1}, ig);	Edible oil (same volume, ig) and normal	(1) NFS (ZL score, 3, 7, 10, and 14 d) (2) NFS (balance	(1) $P < 0.05$ (2) $P < 0.05$ (3) $P < 0.01$

				(350 mg kg^{-1})	2 d after occlusion; once daily for 12 d	saline (same volume, ip); 2 d after occlusion; once daily for 12 d	beam-walking test, 3, 7, 10, and 14 d) (3) NFS (adhesive removal test, 3,7,10, and 14 d) (4) NFS (bar-grasping test, 3, 7, 10, and 14 d) (5) Normal neuron count (6) Nissl body iod (7) IL-6 content (8) IL-10 content (9) NF-kBp65 content (10) Cerebral cortex ultrastructure	(4) $P < 0.01$ (5) $P < 0.05$ (6) $P < 0.05$ (7) $P < 0.01$ (8) $P > 0.05$ (9) $P > 0.05$ (10) NR
26.	Tan Lingli, 2014	SD rats (NR, 6/6), 200 – 220 g	Permanent MCAO	3.5% chloral hydrate	Catalpol (5 mg kg^{-1}, ip); 6 h after occlusion; once daily for 7 d	Normal saline (same volume, ip); 6 h after occlusion; once daily for 7 d	(1) NFS (balance beam-walking test, 1, 4, 7, and 14 d) (2) NFS (cylinder test, 1, 4, 7, and 14 d) (3) Vessel length (4) Neuron count (5) Glial cell count (6) Cell morphology	(1) $P < 0.01$ (2) $P < 0.01$ (3) $P < 0.01$ (4) $P < 0.05$ (5) $P < 0.05$ (6) NR
27.	Lui et al. 2011	SD rats (male, 12/14), 260 – 290 g	Permanent MCAO	10% chloral hydrate (350 mg kg^{-1})	Catalpol (15, 30, and 60 mg kg^{-1}, ig); 2 d after occlusion; once daily for 12 d	Distilled water (same volume, ig); 2 d after occlusion; once daily for 12 d	(1) NFS (ZL score, 3,6, 9, 12, and 14 d) (2) NFS (balance beam-walking test, 3, 9, and 14 d) (3) NFS (adhesive removal test, 3, 9, and 14 d) (4) Lactic acid content (5) Pyruvic acid content (6) Lactic acid/pyruvic acid (7) Na^{+}, K^{+}-ATPase activity (8) Ca^{2+}, Mg^{2+}-ATPase activity	(1) $P < 0.05$ (2) $P < 0.05$ (3) $P < 0.01$ (4) $P < 0.05$ (5) $P < 0.05$ (6) $P < 0.01$ (7) $P < 0.01$ (8) $P < 0.01$
28.	Zhang Xiaoshuang, 2016	SD rats (male, 10/10), 200 – 250 g	Permanent MCAO	Chloral hydrate (300 mg kg^{-1})	Catalpol (15, 30, 60 mg kg^{-1}, ig); 3 d after occlusion; once daily for 12 d	Edible oil (same volume, ig) and normal saline (same volume, ip); 3 d after occlusion; once daily for	(1) NFS (ZL score, 3, 7, 10, and 14 d) (2) LFB IOD (3) MBP IOD (4) Brain pathohistology	(1) $P < 0.05$ (2) $P < 0.01$ (3) $P < 0.01$ (4) NR

						12 d		
29.	Wan Dong et al, 2016	SD rats (male, 9/9), 220–250 g	Permanent MCAO	Chloral hydrate	Catalpol (5 mg kg^{-1}, ip); 1 d after occlusion; once daily for 7 d	MCAO without any intervention	(1) NFS (cylinder test, 1, 4, 7, and 15 d) (2) NFS (foot-fault test, 1, 4, 7, and 15 d) (3) Cerebral blood flow ratio (4) VWF-PCNA colocalization number (5) VWF-PCNA colocalization area IOD (6) pSTAT3 translocation number (7) pSTAT3-positive cell IOD (8) EPO/NADPH IOD (9) EPOR/GAPDH IOD (10) pJAK2/NADPH IOD (11) pSTAT3/GAPDH IOD (12) pSTAT3-VEGF DNA-binding activity (13) VEGF mRNA (14) VEGF/NADPH IOD (15) VEGF-positive cell IOD	(1) $P < 0.01$ (2) $P < 0.01$ (3) $P < 0.01$ (4) $P < 0.05$ (5) $P < 0.05$ (6) $P < 0.01$ (7) $P < 0.01$ (8) $P < 0.01$ (9) $P < 0.01$ (10) $P < 0.01$ (11) $P < 0.01$ (12) $P < 0.01$ (13) $P < 0.01$ (14) $P < 0.01$ (15) $P < 0.01$
30.	Zhiming et al, 2017	SD rats (male, 13/13), not mentioned	Permanent MCAO	NR	Catalpol(15mg /kg/d) administered by gavage once a day for 2 months	Sterile distilled water administered by gavage once a day for 2 months	(1) NFS (Open field Test) (2) NFS (Discrimination Index in Novel Object Recognition Test) (3) Expression of dynamin 1, PSD-95, and synaptophysin in the Cerebral cortex and Hippocampus (4) Neurite length (immunocytochemistry staining)	(1)P>0.05 (2)P<0.05 (2)P<0.05 P<0.01 (3)P<0.01
31.	Wang	SD rats (male,	Perman	isoflura	Catalpol for 14	Saline solution	(1) NFS	(1) P<0.05

	et al, 2021	30/30) 180-220g	ent MCAO	ne	days (2.5, 5.0, 10.0 mg kg^{-1},ip) after occlusion, once daily		(neurological deficit score, 0, 7, 14 d) (2) IV (TTC staining) (3) Recovery of neuron layout and increased axonal length (4) neurogenesis and angiogenesis (5) VEGF-PI3K/AKT and VEGF-MEK1/2-ERK1/2 pathways activation (6) improved barrier function of OGD-exposed 3D NVU (7) VEGF-PI3K/AKT and -MEK1/2/ERK1/2 pathways activation	(2) P<0.05 (3) P<0.05, P<0.01 (4) P<0.05, P<0.01 (5) P<0.05, P<0.01 (6) P<0.05, P<0.01 (7) P<0.05, P<0.01
32.	Jinghui Wang et al, 2022	SD rats (male, 12/12), 220–250 g	Permanent MCAO	NR	Catalpol(10mg/kg, intranasal) for 3 d	Sham treated	(1) NFS (Modified neurological severity scores at 24h, 48h and 72h) (2) IV (TTC, 3d) (3) Brain Water content Analysis (4) Cell apoptosis, relative ratio of Bcl-2/ β-actin and Bax/ β-actin (5) Oxidative stress - MDA Content, SOD content (6) Expression of protein Nrf2 (7) Expression of protein HO-1	(1) P<0.01 (2) P<0.01 (3) P<0.01 (4) P<0.01, P<0.05, p<0.05 (5) P<0.05, P<0.05 (6) P<0.05, P<0.01 (7) P<0.01
33.	Hui Feng Zhu et al, 2019	SD rats (male, 27/18), 220–250 g	Permanent MCAO	NR	Catalpol dissolved in physiological saline and administered 6 h after pMCAO, then administered daily for 7 d at doses of 5 or	physiological saline (100 g/mL, i.e.)	(1) NFS (Horizontal ladder test, 1, 3 ,7 d) (2) No. of Nestin- and BrdU-immunoreactive cells in brain cortex, Nestin and BrdU co-expression (3) No. and mean density of Nestin/	(1) P<0.05, P<0.01 (2) P<0.01, P<0.01 (3) P<0.05 (4) P<0.05 (5) P<0.05 (6) P<0.05 (7) P<0.01

					10 mg/kg		BrdU positive cells (4) No. and mean density of Tuj-1/BrdU -positive cells (5) Count of Tuj-1 and Cleaved-caspase 3 cells (6) BDNF expression (7) Nissl body staining	
34.	Shao Yali et al, 2020	SD rats	Permanent MCAO	NR	Catalpol(5 mg/kg, 10 mg/kg, ip)	normal saline	(1) NFS (Bederson score 1, 4, 7, 14, 21, 28 d) (2) NFS (neurological deficit score, Mnss, 1, 4, 7, 14, 21, 28 d) (3) NFS (muscle strength test score,1, 4, 7, 14, 21, 28 d) (4) Number of astrocytes (5) Number of neural stem cells and neurons (6) Expression of DCX (7) Expression of GFAP (8) Expression of Nestin and Neu-N	(1) P<0.05 (2) P<0.05 (3) P<0.05 (4) P<0.05 (5) P<0.05 (6) P<0.05 (7) P<0.05 (8) P<0.05
35.	Huang tao et al, 2019	SD rats (20/20)	Temporary MCAO / 2h	NR	Catalpol(10mg/kg or 20mg/kg, ip)		(1) NFS (neurological deficit score, mNSS) (2) Decrease in levels of MDA, ROS, IL-1β, IL-6, TNF-α, tNOS, i NOS and NO content and Bax protein expression (3) Increase in levels of SOD and GPX content and the Bcl-2 protein expression level	(1) P<0.01 (2) P<0.01 (3) P<0.01
36.	Wandong et al, 2012	SD rats (19/19)	Permanent MCAO	NR	Catalpol (1, 5, and 10 mg·kg-1, i.p) 24 hrs after occlusion,	Normal saline (same volume, ip); 24 hrs after occlusion; once	(1) NFS (Corner Test, 1, 4, 7 and 15 days) (2) IV (magnetic	(1) P<0.05 (2) P>0.05 (3) P<0.05 (4) P<0.05

					once daily for 7 d	daily for 7 d	resonance imaging at 1 and 15 days) (3) dendritic changes of pyramidal neurons in the PIC area (4) synaptophysin p38 protein expression in PIC area	
37.	Wandong et al, 2013	SD rats (10/10)	Permanent MCAO	NR	Catalpol (5mg kg^{-1}, ip) 24 hrs after occlusion, once daily for 7 days	Normal saline (same volume, ip) 24 hrs after occlusion, once daily for 7 days	(1) NFS (Adhesive removal test, 7, 14, 21, 28 days) (2) NFS (Foot fault test, 7, 14, 21, 28 days) (3) NFS (sensorimotor function test, 28 days) (4) IV(MRI at 1 and 28 days) (5) percentage of CST translateralfibres in the unaffected side of the cervical enlargement area of spinal cord (6) colocalization signal of BDA/GAP-43 in cervical enlargement area	(1)P<0.05 (2)P<0.05 (3)P<0.05 (4)P>0.05 (5)P<0.05 (6)P<0.05

3.4 Possible Mechanisms of Neuroprotection by Catalpol

There were ten included articles that examined the neuroprotective effects of Catalpol on ischemic stroke. These articles were summarized as follows: (1) decrease in oxidative reactions through increased SOD, GSH-PX, and catalase activity, increased NOX2 expression, and decreased MDA and NO concentrations [24]; (2) apoptosis inhibition through increased bcl-2 expression and decreased cleaved caspase-3, caspase-9, and Bax expression [24, 22]; (3) suppression of inflammatory responses through IL10 expression reduction [24], (4) suppression of autophagy through LC3 expression [21], and (5) alleviation of energy exhaustion through lactic acid reduction, pyruvic acid reduction, and enhanced activity of Na+, K+-ATPase, and Ca2+, Mg2+-ATPase(6).“Promoting the survival, reparation and regeneration of neural cells by enhancing the expression of NGF, VEGF, BDNF, bFGF, TrKA, TrkB, AKt, and PI3K, thus escalating the mRNA levels of NGF, BDNF, TrKA, and AKt, and decreasing CDNF expression and PI3K mRNA [24, 36, 28]; (7) enhancement of angiogenesis by increased expression of EPO, EPOR, VEGF, JAK2, pJAK2, STAT3, and Ang-1” [17, 19, 26, 29].

4. DISCUSSION

4.1 Efficacy of Catalpol

This is the first systematic review that we are aware of that looked into the effectiveness of Catalpol for experimental acute focal ischemic stroke. Our review of 21 studies involving 805 animals revealed that Catalpol enhanced NFS and significantly decreased IV, pointing to possible neuroprotective effects in experimental acute focal ischemic stroke. Nonetheless, the present study's overall available evidence should be interpreted with caution due to its methodological flaws.

4.2 Implications

The inadequate design of animal research is well documented [40], and it is thought to be a barrier to the development of promising preclinical drug treatments for human disease [41]. “Due to inherent limitations in the primary studies, the included studies in this study are of low quality. Therefore, a number of metrics have been created to either directly or indirectly address methodology quality concerns in animal studies. Reporting guidelines for animal research: reporting in vivo experiments (ARRIVE)” [42] include a 20-item checklist for the Introduction, Methods, Results, and Discussion sections. “To enhance the methodological quality, we advise using the ARRIVE guidelines when planning animal studies on Catalpol. Preclinical studies of acute stroke should follow guidelines on dose, time window, design, outcome assessment, animal species, and model, according to the Stroke Therapy Academic Industry Roundtable (STAIR) meetings” [43]. For the study of Catalpol treatment for experimental stroke, we also recommend applying the STAIR recommendations.

“The failure of numerous medications that appeared promising and demonstrated notable effects in animal studies to be developed into clinical drug treatments is disheartening” [44]. “Two of the primary reasons why drug models have not been successfully translated to human disease are thought to be the use of excessive dosages and the timing of drug administration in animal models, both of which are inappropriate for human disease” [44]. The 21 included studies in this systematic review varied in their initial administration timings and Catalpol dosages in animal models. Therefore, we recommend more research to determine the best gradient dosages and administration schedules in animal models of acute ischemic stroke.

The biological and molecular processes underlying catalpol's neuroprotective benefits are still unclear. The present study showed that Catalpol had neuroprotective effects for ischemic stroke through different mechanisms as follows: (1) reduction of oxidative reactions by increasing the activity of SOD, GSH-PX, and catalase, increasing the expression of NOX2 and decreasing the concentration of MDA and NO; (2) inhibition of apoptosis by increasing bcl-2 expression and decreasing the expression of cleaved caspase-3, caspase-9, and Bax; (3) repression of inflammatory reactions by decreasing the expression of IL10; (4) repression of autophagy by increasing LC3 expression; (5) relief of energy exhaustion by decreasing lactic acid content, increasing pyruvic acid content, and improving the activity of Na+, K+-ATPase and Ca2+, Mg2+-ATPase; (6) promotion of survival, reparation, and regeneration of neural cells through upregulating the expression of VEGF, bFGF, TrKA, TrkB, AKt, and PI3K; (7) enhancement of angiogenesis by upregulating the expression of EPO, EPOR, VEGF, JAK2, pJAK2, STAT3, and Ang-1; (8) neuroprotection through GLP-1R/β-endorphin pathway. Additionally, it has been reported that additional compounds from Radix Rehmanniae possess antioxidation, anti-inflammatory, and antiapoptotic

properties. However, more research is warranted to determine the effectiveness of Catalpol in terms of cellular and molecular alteration mechanisms as well as functional improvement.

Of the 21 included studies, 18 different NFS measuring methods were employed, demonstrating the variety and inconsistency of NFS measuring methods. It is anticipated that more research will be done to determine whether and how the various NFS measurement techniques will impact the findings of animal studies on acute cerebrovascular accidents. The accuracy of various measuring techniques must also be investigated in order to filter the best standards for NFS.

5. LIMITATIONS

Our study has certain limitations that should be taken into account when interpreting it. First, we limited the studies we included to those from English and Chinese databases. Selective bias may be somewhat exacerbated by the lack of research published in other languages [38]. Second, there was limited generalization of the results because only five of the twenty-five studies were published in English, with the rest being all in Chinese.Third, there could be a potential publication bias due to the predominance of studies in Chinese. Fourth, low methodological quality of included studies was indicated by quality scores ranging from 2 to 7 points. The majority of the studies had problems with blinding, randomization, allocation concealment, and sample size calculation—all of which are fundamental requirements for study design [39]. The heterogeneity of animal models and neurobehavioral scoring methods are other methodological concerns of this systematic review.Furthermore, none of the studies that were included used animals with pertinent comorbidities, which would have produced more accurate models of human pathology [39]. Therefore, the current study should therefore be interpreted with caution.

6. CONCLUSION

Catalpol may have neuroprotective effects on experimental acute focal ischemic stroke by lowering oxidative reaction, preventing apoptosis, and suppressing inflammatory responses and autophagy, as the current study showed. It could also improve NFS and lower IV. Catalpol might also be a good option for clinical research. There is a need for rigorous randomized controlled trials to evaluate the efficacy of Catalpol in stroke patients.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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