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Protective effect and possible mechanisms of geniposide for ischemia-reperfusion injury: A systematic review with meta-analysis and network pharmacology of preclinical evidence

Chaoqin Luo [a,1], Lingfeng Wang [a,1], Yifan Wu [a], Menghan Liu [a], Baoxin Chen [b], Yuqiao Lu [c], Yunling Zhang [d], Chen Fu [e,*], Xuemei Liu [c,**]

[a] Beijing University of Chinese Medicine, Beijing, China
[b] Neurology Department, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, China
[c] Office of Academic Research, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, China
[d] Department of Neurology, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, China
[e] Experimental Center of Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, China

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ABSTRACT

Background: Geniposide, as a pharmacologically bioactive component, is derived from a classic and common Chinese herb, *Gardenia jasminoides* Ellis. Geniposide has been shown to be effective for treating I/R injury in recent studies. Current effectively pharmaceutical treatments are scarce, and treatment based on geniposide may become a novel option. As far as we know, this research is the initial systematic evaluation of the protective effects of geniposide in I/R injury.

Aim of the study: This study is engrossed in evaluating the mechanism of action of geniposide in I/R injury through a preclinical systematic review with meta-analysis and network pharmacology.

Materials and methods: We built a systematic review which provided a view of effect and mechanism of geniposide for I/R injury. Based on seven databases, an open-ended search from their inception to August 31st, 2022, was conducted. Animal studies on the effects of geniposide in I/R injury were considered. The data was analyzed using Review Manager 5.3, and bias was assessed using the CAMARADES 10-item scale. 13 articles including 279 animals were selected finally. And network pharmacology was joined to elucidate the mechanism.

Results: According to the meta-analysis, in I/R injury, geniposide can attenuate cardiomyocytes viability and the size of MI, decrease the volume of cerebral infraction and neurological score, decrease serum ALT and AST activity, and downregulated serum Cr and BUN. The review found that geniposide protects against I/R injury by inhibiting apoptosis, oxidation, inflammation and improvement of autophagy and mitochondrial respiration, which is consistent with the results of the network pharmacology screening.

Conclusion: This preclinical systematic review including meta-analysis and network pharmacology, which was the first one summarizing the relationship between geniposide and ischemia diseases, shows a novel therapy for I/R injury and appears an enticing implication of geniposide in I/R injury, and further research is looked forward. Given the restricted quantity of included researches and the unclear risk of bias of the studies, we should interpret the results with caution.

* Corresponding author.
** Corresponding author.
E-mail addresses: fuchen2003@163.com (C. Fu), liuxuemeitcm@163.com (X. Liu).
[1] The authors have contributed equally to this work and share first authorship.

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Abbreviation

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCCA	bilateral common carotid artery
BUN	blood urea nitrogen
CCK-8	Cell Counting Kit-8
CI	confidence interval
CNKI	Chinese National Knowledge Infrastructure
Cr	serum creatinine
FS	fractional shortening
I/R	Ischemia-reperfusion
IVSs	interventricular septal thickness
LAD	left anterior descending coronary artery
LVEF	left ventricular ejection fraction
MCA	middle cerebral artery
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2Htetrazolium bromide
PCI	percutaneous coronary intervention
PPI	protein-protein interaction
rt-PA	recombinant tissue plasminogen activator
SD	Sprague-Dawley
SinoMed	Chinese Biomedical Literature Database
SMD	standard mean difference
SV	stroke volume
TCMSP	The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

1. Introduction

The occurrence of ischemia-reperfusion (I/R) injury happens when blood flow is restored to ischemic tissues [1] and contributes to serious clinical diseases with high morbidity and mortality, such as acute coronary syndrome, ischemic stroke, acute kidney injury, trauma, circulatory arrest, and peripheral vascular disease [2]. The I/R injury mechanisms involve the molecular, cellular, tissue to whole organism levels which all experience a series of cell and organ damage [3] and mainly feature inflammation, oxidative stress, apoptosis, energy metabolism disorder, microvascular dysfunction, and leucocyte-endothelial cell adhesion [4]. The negative impact of I/R injury involves many diseases, especially cardiovascular and cerebrovascular diseases which are on account for ischemic heart disease and stroke [4]. Stroke is a leading contributor of morbidity and disability globally [5]. In 2019, the number of stroke incident cases was 12.2 million, the number of prevalent cases was 101 million, and the number of stroke deaths was terribly 6.55 million [6]. And importantly, 62.4% of all new strokes were ischemic stroke. So far, recombinant tissue plasminogen activator (rt-PA), which is used in the treatment of ischemic stroke and get the target of rapid reperfusion, is still the only drug in practice [7]. Nevertheless, considering the insufficient duration of the therapeutic period (4.5h) and a part of people who may suffer hemorrhagic transformation, just under 10% of stroke patients can receive rt-PA treatment [8]. The most common type of ischemic heart disease is acute myocardial infarction (AMI), which also ranks as a leading cause in terms of death worldwide [9]. Each year, there are over 7 million cases around the world suffering from AMI which is best treated with primary percutaneous coronary intervention (PCI). PCI can give additional revascularization, but its help is still limited when meeting nonculprit lesions which may represent stable coronary artery plaques [10]. In all, although several clinical therapies are used to treat ischemic diseases induced by I/R injury, their efficacy remains unsatisfactory, and finding efficacious treatments to alleviate the damages of I/R injury is meaningful and highly necessary.

For centuries, China has documented the utilization of herbal remedies to treat cardiovascular and cerebrovascular diseases, including ischemic diseases. *Gardenia jasminoides* Ellis, known as Zhi-Zi in Chinese pharmacopeias, is a classic and common Chinese herb which is widely applied to clear away heat evil and cool blood, and is proved to have effect on anti-inflammation, antioxidant and ischemia brain injury [11,12]. Geniposide, derived from the fruit of *Gardenia jasminoides* Ellis, is a representative type of iridoid glycoside and a pharmacologically bioactive component. Fig. 1 demonstrates the chemical structure of geniposide. Pharmacological research confirms geniposide play an effective role in inhibiting inflammation, pathogenicity, oxidation, and radiation [13,14]. Because of a variety of pharmacological properties, lots of preclinical studies related with diseases affecting the nervous system, cardiovascular system, liver, diabetes,and tumors, have used geniposide as a research target [15]. Since the first study investigating the antithrombotic effect of geniposide and its metabolite genipin in animal model [16], the advantages of geniposide have been reported in different animal I/R injury models. Recent study has shown that geniposide can protect neurons against I/R injury by activating autophagy and inhibiting inflammation [17]. Other study revealed that geniposide can suppress oxidative stress, so as to reduce myocardial I/R injury in diabetic rats [18]. As previously mentioned, geniposide has been showed as a possible medicine for I/R injury treatment, and the preclinical results are promising. However, the underlying mechanisms of geniposide on I/R injury have not been systematically analyzed.

However, geniposide are not yet clinically available for the treatment of I/R injury, and the underlying mechanisms of geniposide

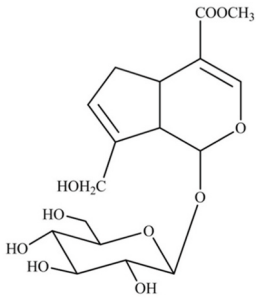


Fig. 1. Geniposide's chemical structure.

on I/R injury have not been systematically analyzed. A full review of the limitations and potential of all available preclinical evidence would be beneficial for the translation of a new therapeutic from experimental results to the clinic, and development of new form of medication such as nanocomposites [19], as well as reveal hidden innovative strategies and potential limitations in animal experiments. Therefore, we carried out this systematic review which included meta-analysis and network pharmacology to summarize the efficacy of geniposide in the I/R injury treatment in preclinical studies，and provide a view of effect and mechanism of geniposide for I/R injury. The findings of our study will provide a theoretical foundation and clinical guidelines for applying geniposide therapy to I/R injury. As far as we know, this study is the initial systematic evaluation of the protective effects of geniposide in I/R injury.

2. Materials and methods

2.1. Search strategy

The review strictly followed the PRISMA guidelines [20], based on previously prepared, unregistered protocol. Two reviewers independently screened all citations, selected studies and evaluated the risk of bias. I/R injury studies with geniposide on animals were identified based on PubMed, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), Wanfang Data Information Site, VIP information database, and Chinese Biomedical Literature Database (SinoMed). A comprehensive search was conducted from their inception to August 31st, 2022. We chose the following terms in English and Chinese to search animal-related studies: ("geniposide") AND ("ischemic" OR "reperfusion" OR "infarction" OR "ischemia" OR "ischemia").

2.2. Eligibility criteria

Two researchers analyzed all the studies independently in order to determine the study's eligibility for inclusion in the review. Inclusion criteria consisted of the following: 1) animal models that were induced with I/R injury using different methods; 2) treatment group were treated only with the geniposide; 3) control group were treated with vehicle, nonfunctional liquids of equal volume or no treatment; 4) the primary outcomes: myocardial I/R injury biomarkers (i.e. MI size, LVEF, FS, IVSs), cerebral I/R injury biomarkers (i.e. volume of cerebral infraction, neurological score), liver I/R injury biomarkers (i.e. ALT, AST), renal I/R injury biomarkers (i.e. Cr, BUN); 5) the secondary outcomes: experimental mechanism indexes of geniposide treatment in animals.

Exclusion criteria were as follows: 1) combinations of geniposide and other medications; 2) no available or complete data; 3) not animal research (i.e. clinical trials, case reports, cell researches), review articles, comments, and abstracts; 4) not an I/R injury model; 5) duplicate publication; 6) no control group.

2.3. Data extraction

Two independent researchers finished a quality assessment and data extraction form which collected the following information from all included preclinical studies: 1) first author, year of publication; 2) animal details (i.e. species, sex, number, weight); 3) anesthetics use (i.e. types, administration method, dosage); 4) drugs in treatment and control groups (i.e. administration method, dosage, treatment duration); 5) outcomes index and intergroup differences.

For avoiding confusion due to the different time points or diverse doses of the drug in experimental animals, we built a unified standard that just chose the final time point and the maximum dose to record. Because of a part of incomplete data, we contacted the authors for accessing supplementary data.

2.4. Risk of bias in individual studies

The methodological quality of animal experiments was assessed by the SYRCLE's Risk of Bias tool for animal researches [21]. Two independent researchers (CQL and LFW) evaluated the risk of bias and any disagreement was resolved by the third researcher (CF).

2.5. Statistical analysis

The researcher finished the detailed statistical analysis which runs through this paper by RevMan 5.3. software (https://community.cochrane.org). Standard mean difference (SMD) with a 95% confidence interval (CI) was utilized to express the outcomes of continuous variables. To assess the heterogeneity of the studies, the Cochrane's Q test ($P < 0.05$ indicating statistical significance) and the I^2 statistical test were utilized. If $I^2 > 50\%$, a random effects model will be utilized. If $I^2 < 50\%$, a fixed-effect model will be used. Subgroup analysis was conducted when needed. Sensitivity analysis was implemented to improve the results robustness.

2.6. Network target analysis

To screen the target library of geniposide, the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) were applied. From the TTD (http://bidd.nus.edu.sg/group/cjttd/) databases, DisGeNET (http://www.disgenet.org/) databases and GeneCards (https://www.genecards.org/) databases, relevant protein targets of I/R injury were collected and a library of I/R injury was established. Uniprot (https://www.uniprot.org) database was used to standardize both protein target information of geniposide and I/R injury. By comparing the targets of geniposide with relevant targets of I/R injury, researchers establish a database of the intersection of the above-mentioned targets. Then, String 11.5 (https://cn.string-db.org/) database and Cytoscape 3.8.2 software were employed to analyze the interaction of protein targets from geniposide and I/R injury, and built a protein-protein interaction (PPI) network model.

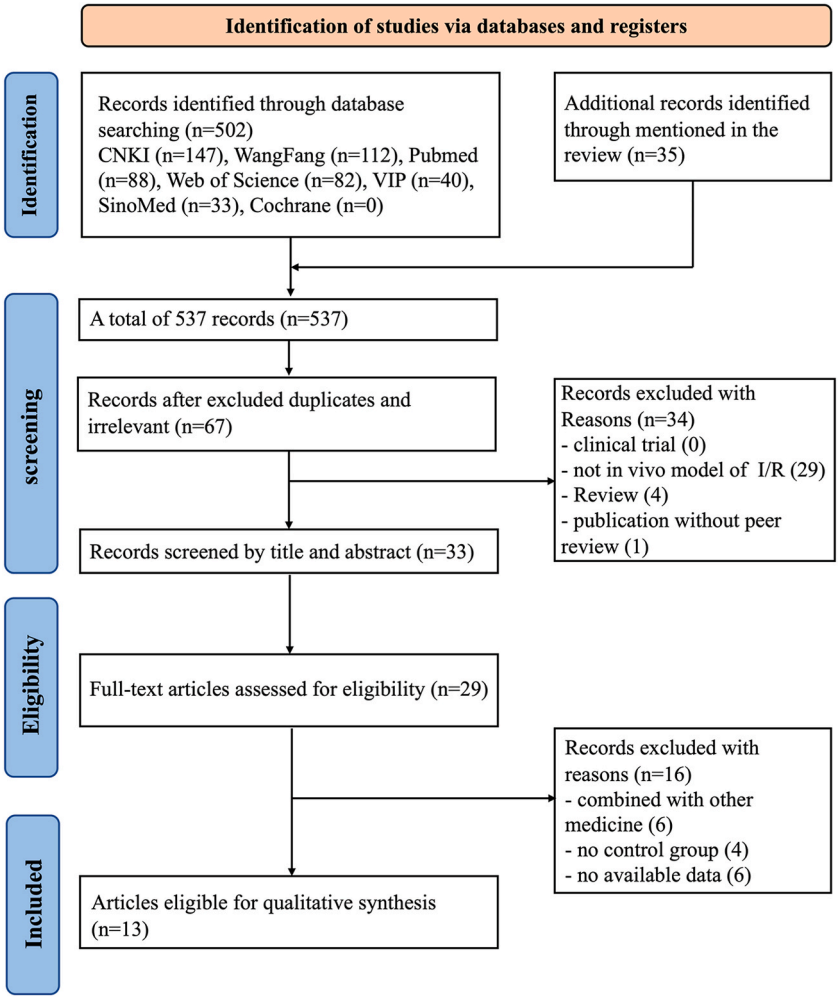


Fig. 2. Flow diagram of systematic literature search.

Table 1
Characteristics of the 13 included animal studies.

Study (years)	Species (sex; n = experimental/ control group)	Weight	Model(method)	Anesthetic	Treatment group (Method to astraddle sides)	Control group	Outcome index (time)	Intergroup differences
Zhanjun Zhang,2006	SD rats (male; 22/ 22)	300–350g	MCAO	70% N_2O and 2% halothane (the balance O_2)	Geniposide (15 mg/ kg) for 1.5h before the ischemia	Intravenous infusion of the equal volume of normal saline	1. Behavior Score 2. Cerebral infarct volume 3. P-CREB 4. CREB 5. Akt	1. $P > 0.05$ 2. $P < 0.05$ 3. $P > 0.05$ 4. $P > 0.05$ 5. $P > 0.05$
Shiwan Duan,2012	SD rats (male; 15/ 15)	250–280g	MCAO	Chloral hydrate (300 mg/kg; i.p.)	Geniposide (60 mg/ kg) for 4 days after ischemia	Given the same amount of 1% Tween solution	1. Neurological deficit score 2. COX-2 activity 3. 5-LOX activity	1. $P > 0.05$ 2. $P < 0.01$ 3. $P < 0.01$
Joonki Kim,2013	C57BL/6 mice (male; 8/8)	23–25g	Clamping the left branches of the portal vein and hepatic artery (ischemia; 60min; reperfusion; 6h)	ketamine (55 mg/kg body weight), xylazine (7 mg/kg body weight)	Geniposide (20 mg/ kg; p.o.) before ischemia	Given the same amount of saline	1. Serum ALT 2. Serum AST 3. MDA 4. Ratio of GSH/GSSG 5. HO-1 protein 6. The number of TUNEL-positive hepatocytes 7. Caspase-3 activity 8. Tbid 9. Cytochrome *c* protein	1. $P < 0.05$ 2. $P < 0.05$ 3. $P < 0.05$ 4. $P < 0.05$ 5. $P < 0.05$ 6. $P < 0.01$ 7. $P < 0.05$ 8. $P < 0.05$ 9. $P < 0.05$
Baosheng Huang,2017	SD rats (male; 8/ 8)	280–320g	MCAO	Chloral hydrate(0.3 mg/kg)	Geniposide (75 mg/ kg; i.p.) for 3 h after ischemia	Given the same amount of saline	1. Infarct volume 2. Neurological severity score 3. Neurons in DG 4. Ratio of Bcl-2/Bax 5. Evans blue 6. Haemorrhage volume 7. GluN2A 8. GluN2B 9. P-AKT 10. P-ERK 11. PSD-95	1. $P < 0.01$ 2. $P < 0.01$ 3. $P < 0.05$ 4. $P < 0.05$ 5. $P < 0.05$ 6. $P < 0.05$ 7. $P < 0.05$ 8. $P > 0.05$ 9. $P < 0.05$ 10. $P < 0.05$ 11. $P < 0.05$
Y.-P. Rong,2017	SD rats (male; 10/ 10)	200–240g	isolate the hepatic arterial vein, hepatic portal vein, and hepatic duct (ischemia; 30 min; reperfusion; 6h)	3% sodium (30 mg/ kg)	Geniposide (20 mg/ kg; i.p.) for 3 h after ischemia and for 30min before the ischemia	Given the same amount of saline	1. Serum ALT 2. Serum AST 3. LDH 4. Damage Score 5. IL-6 6. MCP-1 7. TNF-α 8. Bax 9. BCL-2 10. PI3K 11. P-AKT 12. mTOR	1. $P < 0.01$ 2. $P < 0.01$ 3. $P < 0.01$ 4. $P < 0.01$ 5. $P < 0.05$ 6. $P < 0.05$ 7. $P < 0.05$ 8. $P < 0.05$ 9. $P < 0.05$ 10. $P < 0.05$ 11. $P < 0.05$ 12. $P < 0.05$
Haiyan Zhang,2017	SD rats (male; 14/ 14)	260–300g	2-VO	10%Chloral hydrate (350 mg/kg)	Geniposide (50 mg/ kg/day) for 30 days after ischemia	Given the same amount of saline	1. Morris water maze test(Escape latency) 2. The ratio of dead cells in hippocampal CA1 3. The ratio of dead cells in the external granular layer of the cerebral cortex 4. The ratio of dead cells in the internal pyramidal layer of cerebral cortex 5. The ratio of dead cells in the cingulate cortex 6. AchE 7. NOS 8. SOD 9. MDA	1. $P < 0.05$ 2. $P > 0.05$ 3. $P < 0.05$ 4. $P < 0.05$ 5. $P < 0.01$ 6. $P < 0.01$ 7. $P < 0.01$ 8. $P > 0.05$ 9. $P > 0.05$
Fei Chen,2017	C57 mice (male; 10/10)	15–25g	clamping the left kidney pedicle (ischemia; 1h; reperfusion; 24h)	1%sodium pentobarbital (40 mg/kg)	Geniposide (20 mg/ kg; i.g.) for 8 days after ischemia	Given the same amount of saline	1. Cr 2. BUN 3. Renal injury score 4. MDA 5. SOD 6. CAT 7. GPx 8. TNF-α 9. IL-6 10. IL-1β 11. NF-κB	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. $P < 0.05$ 9. $P < 0.05$ 10. $P < 0.05$ 11. $P < 0.05$
Tian Shen,2017	SD rats (male; 7/ 8)	250–280g	MCAO	no report	Geniposide (60 mg/ kg/day; i.g.) for 4 days after ischemia	Given the same amount of 1% Tween solution	1. Neurological deficit score 2. COX-2 activity 3. 5-LOX activity	1. $P > 0.05$ 2. $P > 0.05$ 3. $P > 0.05$

(*continued on next page*)

Table 1 (*continued*)

Study (years)	Species (sex; n = experimental/ control group)	Weight	Model(method)	Anesthetic	Treatment group (Method to astraddle sides)	Control group	Outcome index (time)	Intergroup differences
Lijuan Li,2020	SD rats (male; 12/ 12)	350–400g	2-VO	3.5%Chloral hydrate	Geniposide (100 mg/kg/day; i.g.) for 4 weeks after ischemia	Given the same amount of saline	1. Behavioral test(Morris water maze) 2. Behavioral test(space exploration) (Percent time in target quadrant) 3. Behavioral test(Visual platform experiment) 4. Expression of GFAP in the frontal lobe and in the hippocampus 5. Expression of iNOS and NF-κB in the frontal lobe and in the hippocampus 6. TNF-α、IL-6	1. $P < 0.05$ 2. $P < 0.05$ 3. $P > 0.05$ 4. $P < 0.05$ 5. $P < 0.05$ 6. $P < 0.05$
Xuexiu Luo,2020	SD rats (male; 12/ 12)	170–220g	Block the left anterior descending (LAD) coronary artery (ischemia; 30min; reperfusion; 2h)	1% pentobarbital sodium (50 mg/kg)	Geniposide (100 mg/kg; p.o.) for 30 min before ischemia	Given the same amount of saline	1. Myocardial infarct size 2. EF 3. FS 4. SV 5. IVSs 6. The number of TUNEL positive cell 7. Ratio of Bax/GAPDH 8. Ratio of Bcl-2/GAPDH 9. Ratio of Bcl-2/Bax 10. ATG5 11. ATG7 12. Beclin1 13. Ratio of LC3-II/LC3-I 14. P62 15. P-AKT	1. $P < 0.01$ 2. $P < 0.01$ 3. $P < 0.01$ 4. $P < 0.01$ 5. $P < 0.01$ 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. $P < 0.01$
Yun Wang,2021	SD rats(male; 6/ 6)	250–270g	MCAO	10%Chloral hydrate (400 mg/kg, i.p.)	Geniposide (60 mg/ kg; p.o.) for 30 min before ischemia	Given the same amount of saline	1. Neurological scores 2. State 3 respiration 3. State 4 respiration 4. RCR 5. ADP/O 6. OPR	1. $P < 0.01$ 2. $P < 0.01$ 3. $P > 0.05$ 4. $P < 0.01$ 5. $P > 0.05$ 6. $P < 0.01$
Jun Wang,2012	SD rats(male; 6/ 6)	–	MCAO	10%Chloral hydrate (350 mg/kg, i.p.)	Geniposide (60 mg/ kg; i.v.)	No treatment	1. The percentage of cerebral infarction 2. Cell viability 3. TNF-α 4. IL-1β 5. IL-6 6. IL-8 7. IL-10 8. TLR4 mRNA 9. TLR4 10. Ratio of *p*-ERK1/2 11. P-IκB 12. P-p38 13. NF-κB p65	1. $P < 0.01$ 2. $P < 0.01$ 3. $P < 0.01$ 4. $P < 0.01$ 5. $P < 0.01$ 6. $P < 0.01$ 7. $P < 0.01$ 8. $P < 0.05$ 9. $P < 0.05$ 10. $P < 0.05$ 11. $P < 0.01$ 12. $P < 0.05$ 13. $P < 0.05$
Haiyan Li2022	C57BL/6 mice (male; 6/6)	20–25g	Block the left anterior descending (LAD) coronary artery (ischemia; 45min; reperfusion; 4h)	pentobarbital sodium (40 mg/kg)	Geniposide (20 mg/ kg; i.g.)	Given the same amount of saline	1.LVEF, LVFS,LVEDd, LVESd 2.serum LDH,CK,CK-MB 3.myocardial infarction size 4.relative fold of *p*-AMPK/AMPK, relative fold of *p*-ACC, relative fluorescence level 5.SOD2 activity 6.NLRP3 mRNA,ASC mRNA,IL-1β mRNA,IL-18mRNA relative expression	1. $P < 0.01$ 2. $P < 0.013$. $P < 0.05$ 4. $P < 0.01$ 5. $P < 0.05$ 6. $P < 0.01$

3. Results

3.1. Study selection

The flowchart summarizing the literature search process is shown in Fig. 2. For preclinical I/R injury studies, a total of 537 articles were found, and 67 articles were preserved after excluded duplications and irrelevant articles. Next, after examining the headings and abstracts of every article, 33 studies were isolated. Then, of the 29 studies which can be assessed by full text screening, 13 papers [22–33] were eligible for inclusion in this review; other 16 studies were excluded.

3.2. Characteristics of included studies

The data characteristics from 13 studies are listed in Table 1. Thirteen animal researches between 2006 and 2022 were included. Among them, five experiments [22,23,26,28,31] were published in Chinese, and eight experiments [24,25,27,29,30,32,33]were published in English. Healthy adult Sprague-Dawley (SD) male rats were used in ten studies [22–24,26–32], whereas three studies [25,26,33]used C57BL/6 mice. During the studies, the rats weighed from 170g to 400g. Six studies used chloral hydrate to induce anesthesia [23,24,27,29,31,32]; 70% N_2O and 2% halothane were used in one study [22]; one study used ketamine and xylazine; one study used sodium [27]; three studies used sodium pentobarbital [26,30,33]; and one study did not state the anesthetic used [28].To develop cerebral I/R injury models, seven studies used occlusion of the middle cerebral artery (MCA) [22–24,27,28,32,34]; and two studies used permanent ligation of the bilateral common carotid artery(BCCA) [29,31]. Two studies [30,33] ligated the left anterior descending coronary artery (LAD) to induce the myocardial I/R model. One study [26] clamped the left kidney pedicle to induce renal I/R model. To establish hepatic I/R injury model, one study [23] clamped the left branches of the portal vein and hepatic artery, while one study [27] isolated the hepatic arterial vein, hepatic portal vein, and hepatic duct. For outcome measures, the volume of cerebral infarction was used in four studies [22,24,27,30], MI size in one study [30], neurological score in three studies [22,27,32], serum aminotransferase activity in two studies, serum creatinine and usea nitrogen in one study [26]. Furthermore, thirteen studies [22–33] reported the chemical analysis of geniposide. The characteristics of geniposide were shown in Table 2.

SD rats, Sprague-Dawley rats; MCAO, middle cerebral artery occlusion; CREB, cyclic AMP response element binding protein; P-CREB, hosphor-cyclic AMP response element binding protein; Akt, protein kinase B; COX-2,cyclooxygenase-2; 5-LOX,5-lipoxygenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MDA, malondialdehyde; GSH, glutathione; GSSG, oxidized GSH; HO-1, heme oxygenase-1; TUNEL, TdT-mediated dUTP nick-end labeling; Tbid, truncated BH3 interacting domain death agonist; VEGF, vascular endothelial growth factor; ANG-1, angiopoietin-1; P-ERK, hosphor-extracellular signal-regulated kinases; PSD-95, post-synaptic density protein-95; LDH, lactate dehydrogenase; IL-6, interleukin-6; MCP-1, monocyte chemoat-tractant protein-1; TNF-α, tumor necrosis factor-α; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma-2; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; AchE, Acetylcholin Esterase; NOS, Nitric Oxide Synthase; SOD, superoxide dismutase; Cr, creatinine; BUN, blood urea nitrogen; CAT, catalase; GPx, glutathione peroxidase; IL-1β, interleukin-1β; NF-κB, nuclear factor-κB; GFAP, glial fibrillary acidic hospho; iNOS, inducible nitric oxide synthase; EF, ejection fraction; FS, fractional shortening; SV, stroke volume; IVSs, interventricular septal thickness; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ATG, antithymocyte globulin; LC3-I, light

Table 2
Statement of the characteristics of geniposide.

Study	Source	Species, concentration	Quality control reported?	Chemical analysis reported?
Zhanjun Zhang,2006	Beijing University of Traditional Chinese Medicine Pharmaceutical Factory	geniposide, 15 mg/kg	N	N
Shiwan Duan,2012	Shaanxi Zhongxin Biotechnology Co., LTD	geniposide, 60 mg/kg	Y (110831)	Y-HPLC
Jun Wang,2012	the National Institutes for Food and Drug Control, Beijing, China	geniposide, 60 mg/kg	Y (110749–200714)	Y-HPLC
Joonki Kim,2013	ChromaDex Co., Irvine, CA, USA	geniposide, 20 mg/kg	N	N
QIFA YE,2016	China National Pharmaceutical Group Corporation (Beijing, China)	geniposide, 50 mg/kg	N	N
Baosheng Huang,2017	Abcam and Yuanye Shanghai Biotechnology Co., Ltd	geniposide, 75 mg/kg	N	N
Y.-P. Rong,2017	Zeng Lang, Nang Jing	geniposide, 20 mg/kg	N	Y-HPLC
Haiyan Zhang,2017	Chinese Institutes for Food and Drug Contro	geniposide, 50 mg/kg/day	N	N
Fei Chen,2017	Sigma,USA	geniposide, 20 mg/kg	N	N
Tian Shen,2017	Shaanxi Zhongxin Biotechnology Co., LTD	geniposide, 60 mg/kg/day	Y (110831)	Y-HPLC
Lijuan Li,2020	Chengdu Gemobtyledon Biotechnology Company	geniposide, 100 mg/kg/day	Y(SZY071229)	Y-HPLC
Xuexiu Luo,2020	the National Institutes for Food and Drug Control, Beijing, China	geniposide, 100 mg/kg	Y(MW:388.37)	Y-HPLC
Yun Wang,2021	Chengdu Croma Biological Co., Ltd. (Chengdu, China)	geniposide, 60 mg/kg	N	Y-HPLC
Haiyan Li,2022	Beijing Vital River Laboratory Animal Technology (Beijing, China)	geniposide, 20 mg/kg	N	Y-HPLC

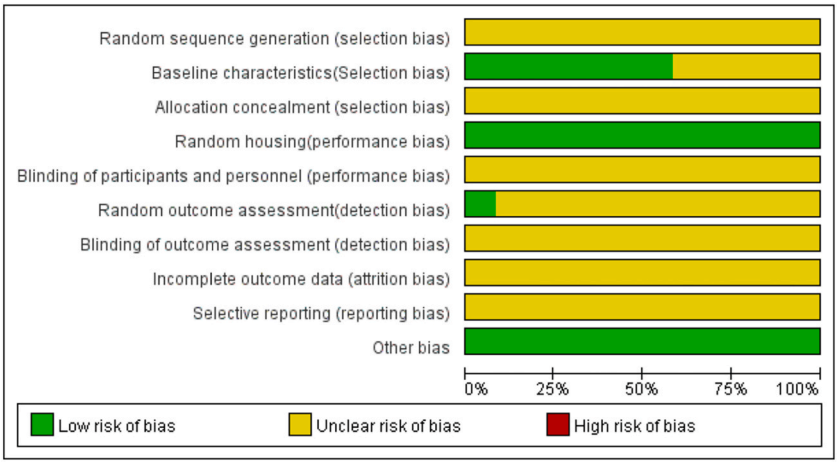
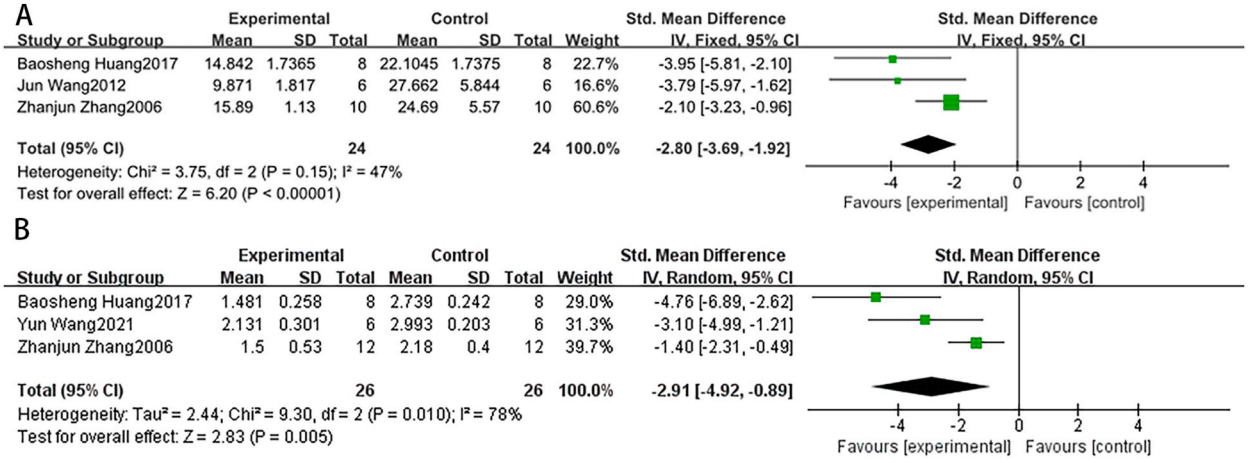


Fig. 3. Risk of bias in 13 included studies.

chain3-I; LC3-II, light chain3-II; RCR, respiratory control ratio; ADP, adenosine diphosphate; O, oxygen; OPR, oxidative phosphorylation rate; IL-8, interleukin-8; IL-10, interleukin-10; TLR4, toll-like receptor 4; P-ERK1, hosphor-extracellular signal-regulated kinases1; P-ERK2, hosphor-extracellular signal-regulated kinases2; *p*-IκB, hosphor-nuclear factor kappa B inhibitor protein; NF-κBp65, nuclear factor-kappa Bp65; LVEF, left ventricular; LVFS, left ventricular, fractional shortening; LVEDd, left ventricular end-diastole diameter; LVESd, left ventricular end-systolic diameter; ASC, apoptosis-associated speck-like protein containing a CARD; AMPK, Adenosine monophosphate-activated protein kinase.

3.3. Study quality

Fig. 3 displays the evaluation of the study quality using SYRCLE 's Risk of Bias tool. All reports only specified "randomized allocation" without providing further information on the randomization method, thus resulting in an "unclear risk of bias." For I/R injury models, seven articles (53.85%) provided details on rat evaluation before inclusion in the experiment to ensure uniform baselines, while there was no mention of this requirement in other articles (As an example, the process of surgery was not stated to be random). In terms of random housing, it was reported that the experimental rats were housed in a random and consistent environment. Only one article (7.69%) tested all the rats in all groups for the meta-analysis's target result, while the other articles were not fully tested and their randomness could not be explained. None of the studies showed details on lost follow-up data or any other deviations, which makes them all judged as "low risk." Moreover, no literature outlined whether allocation was concealed or if those collecting and assessing outcomes or involved in the reporting were blinded. The published literature had some limitations in terms of designing and implementing animal experiment methodology.



Fig, 4. The forest plot: (A) Comparison of the impact of geniposide on cerebral infarction volume to the control group (n = 24 per group). (B) Comparison of the impact of geniposide on neurological scores to the control group (n = 26 per group).

3.4. Effectiveness

3.4.1. Myocardial I/R injury

Cardiomyocyte Apoptosis Rate. One study [30] reported the effect of geniposide on reducing H9c2 cell apoptosis rate was significant compared to the control group ($P < 0.01$).

Cardiomyocytes Viability. One study [30] reported the effect of geniposide on increasing cell viability was significant compared to the control group ($P < 0.01$).

MI Size. One study [33] showed the effect of geniposide on reducing MI size was significant compared to the control group ($P < 0.01$).

3.4.2. Cerebral I/R injury

Cerebral Infraction Volume. Meta-analysis of three studies [22,24,26]demonstrated that a significant reduction in cerebral infraction volume was observed with geniposide compared to control groups [n = 48, MD: −2.80, 95%CI: −3.69 to −1.92, $P < 0.00001$; heterogeneity: $\chi^2 = 3.75$, df = 2 ($P = 0.15$), $I^2 = 47\%$] (Fig. 4A).

Neurological Score. Meta-analysis of three studies [22,26,32] demonstrated that a significant reduction in neurological score was observed with geniposide compared to control groups [n = 52, MD: −1.40, 95%CI: −4.92 to −0.89, $P < 0.001$; heterogeneity: $\chi^2 = 9.30$, df = 2 ($P = 0.010$), $I^2 = 78\%$]. (Fig. 4B).

3.4.3. Hepatic I/R injury

ALT. Meta-analysis of two studies [25,27]showed the effect of geniposide on reducing serum ALT activity was significant compared to the control group[n = 36, MD: = −8.00, 95%CI: −10.34 to −5.65, $P < 0.00001$; heterogeneity: $\chi^2 = 1.12$, df = 1 ($P = 0.29$), $I^2 = 11\%$] (Fig. 5A).

AST. Meta-analysis of two studies [25,27]showed the effect of geniposide on reducing serum AST activity was significant compared to the control group [n = 36, MD: = −8.66, 95%CI: 11.80 to −5.53, $P < 0.00001$; heterogeneity: $\chi^2 = 1.59$, df = 1 ($P = 0.21$), $I^2 = 37\%$] (Fig. 5B).

3.4.4. Renal I/R injury

One study [26] reported that a significant reduction in serum Cr and BUN was observed with geniposide compared to control groups ($P < 0.05$).

3.5. Protection mechanisms

3.5.1. Analysis of anti-inflammatory mechanism

Meta-analysis of two studies [26,27] manifested that geniposide could significantly reduce the serum level of TNF-α [n = 40, SMD: −4.23, 95%CI: −6.49 to −1.98, $P < 0.0001$; heterogeneity: $\chi2 = 3.20$, df = 1($P = 0.07$), $I^2 = 69\%$] (Fig. 6A), and one study [31] showed that geniposide could significantly reduce the level of TNF-α in cerebral tissue ($P < 0.05$). Meta-analysis of two studies [24,35] manifested that geniposide could significantly reduce the level of TNF-α in microglia[n = 34, SMD: −2.36, 95%CI: −3.33 to −1.38, $P < 0.0001$; heterogeneity: $\chi2 = 0.12$, df = 2($P = 0.94$), $I^2 = 0\%$] (Fig. 6B). Meta-analysis of two studies [26,27] showed geniposide had a significant effect on reducing the serum level of IL-6 [n = 40, MD: −4.49, 95%CI: −7.26 to −1.72, $P < 0.001$; heterogeneity: $\chi2 = 4.34$, df = 1 ($P = 0.04$), $I^2 = 77\%$] (Fig. 6C) and one study [32] showed that the effect of geniposide on reducing the level of IL-6 in cerebral tissue was significant compared to the control group ($P < 0.05$). One study [26] revealed that geniposide could significantly reduce the level of IL-1β in renal tissue. One study [24] showed that geniposide could significantly reduce the level of IL-8 and IL-10 in

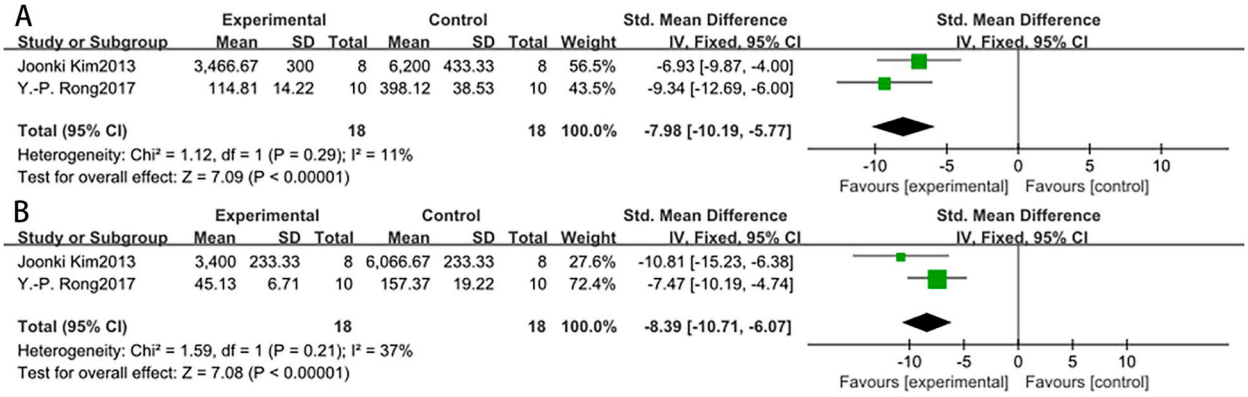


Fig. 5. The forest plot: (A) The forest plot: Comparison of the impact of geniposide on reducing serum ALT to the control group (n = 18 per group). (B) Comparison of the impact of geniposide on reducing serum AST to the control group (n = 18 per group).

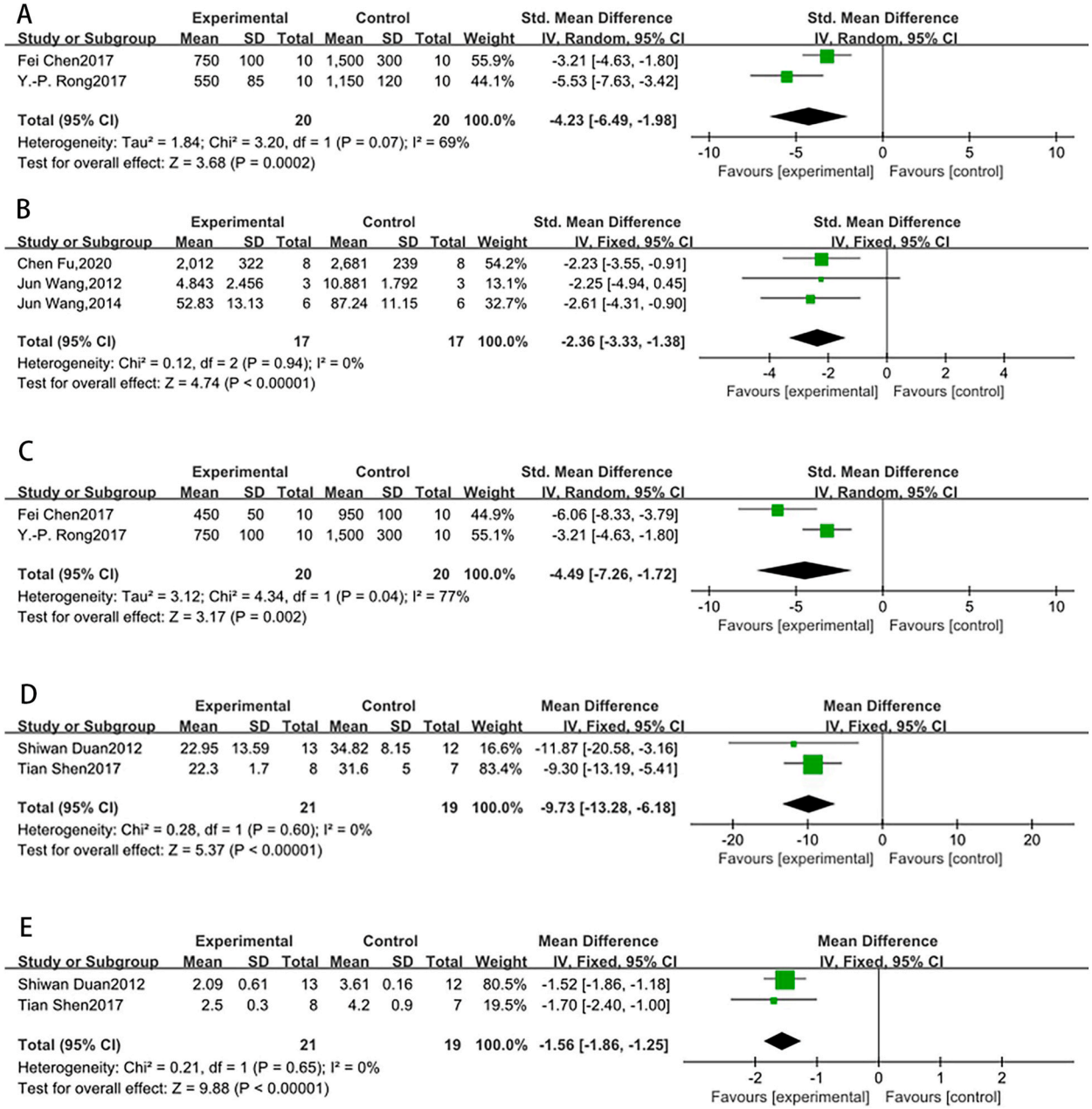


Fig. 6. The forest plot: (A) Comparison of the impact of geniposide on reducing serum TNF-α to the control group (n = 20 per group). (B) Comparison of the impact of geniposide on reducing microglia TNF-α to the control group (n = 17 per group). (C) Comparison of the impact of geniposide on reducing serum IL-6 to the control group (n = 20 per group). (D) The forest plot: Comparison of the impact of geniposide on increasing COX-2 with the control group (experimental group n = 21, control group n = 19). (E) Comparison of the impact of geniposide on reducing 5-LOX with the control group (experimental group n = 21, control group n = 19).

microglia. Meta-analysis of two researches [23,28] showed the effect of geniposide on increasing COX-2 was significant compared to the control group[n = 40, MD: −9.73, 95%CI: −13.28 to −6.18, $P < 0.00001$; heterogeneity: $\chi 2 = 0.28$, df = 1($P = 0.60$), $I^2 = 0\%$] (Fig. 6D).Meta-analysis of two studies [23,28] showed the effect of geniposide on decreasing 5-LOX was significant compared to the control group [n = 40, MD: −1.56, 95%CI: −1.86 to −1.25, $P < 0.00001$; heterogeneity: $\chi 2 = 0.22$, df = 1 ($P = 0.65$), $I^2 = 0\%$] (Fig. 6E).

3.5.2. Analysis of antioxidant mechanism

One study [35] shows the effect of geniposide on reducing the content of NO in microglia was significant ($P < 0.01$). One study

[29] shows geniposide had a significant effect on reducing the content of NOS in cerebral tissue ($P < 0.01$). One study [31] shows geniposide had a significant effect on reducing the content of iNOS in cortex and hippocampus ($P < 0.05$). One study [26] shows the effect of geniposide on increasing the level of SOD in renal tissue was significant ($P < 0.05$). One study [25] shows that geniposide had a significant effect on reducing the level of MDA in hepatic tissue($P < 0.05$).One study [27] shows that the effect of geniposide on reducing the level of MDA in renal tissue was significant ($P < 0.05$). One study [25] shows that the effect of geniposide on increasing the ratio of GSH/GSSG in hepatic tissue was significant ($P < 0.05$).

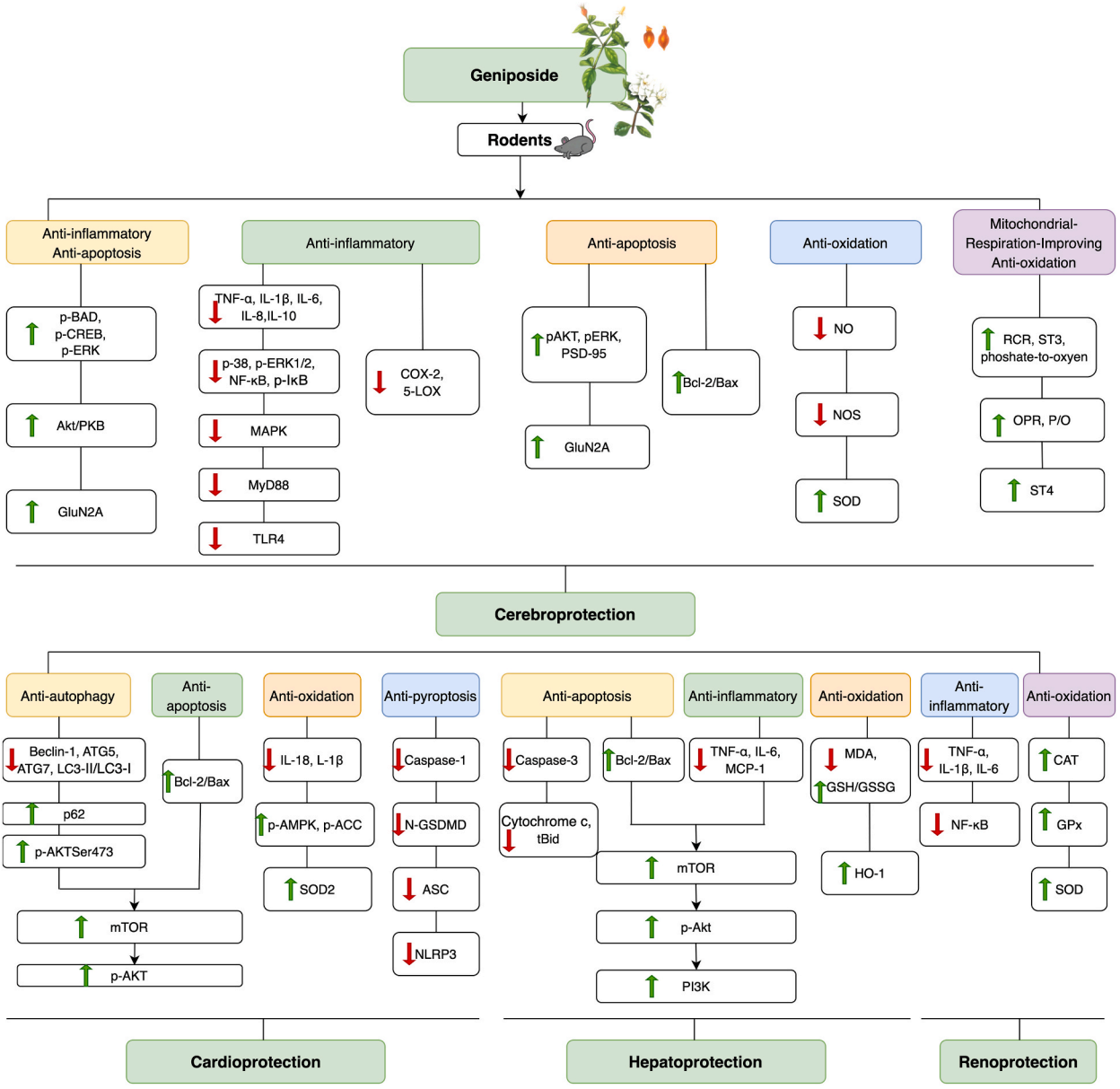


Fig. 7. A panoptic view of molecular mechanisms of geniposide for organs ischemia-reperfusion injury. *p*-ERK, phospho-extracellular signal-regulated kinases; Akt/PKB, Akt-protein kinase B; *p*-CREB, phospho-cAMP response element-binding protein; p-BAD, cytosolic and mitochondrial phospho-Bad; NLRP3, nod-like receptor protein 3; IL-1β, interleukin-1β; TLR4, toll-like receptor 4; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin 1 beta; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; GLP-1R, glucagon-like peptide-1 receptor; Bax, BCL2-associated X protein; Bcl-2, B-cell lymphoma 2; NO, nitric oxide; NOS, nitric oxide synthase; RCR, mitochondrial respiratory control ratio; *p*-AKT, phospho-protein kinases B; mTOR, mammalian target of rapamycin; ATG5, antithymocyte globulin 5; ATG7, antithymocyte globulin 7; PI3K, hosphatidylinositol 3-kinase; MDA, malondialdehyde; tBid, truncated BH3 interacting domain death agonist; HO-1, heme oxygenase-1; GSH/GSSG, glutathione/oxidized GSH; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase.

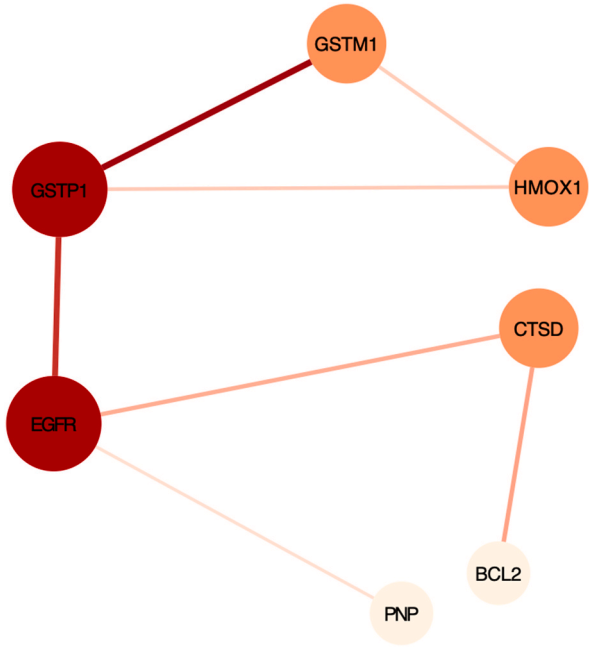


Fig. 8. PPI network from targets of geniposide.

3.5.3. Analysis of anti-apoptosis mechanism

One study [26] shows geniposide had a significant effect on increasing the ratio of Bcl-2/Bax in hippocampus ($P < 0.05$). One study [26] shows geniposide had a significant effect on increasing the ratio of Bcl-2/Bax in heart tissue ($P < 0.01$). One study [27] shows geniposide had a significant effect on increasing the ratio of Bcl-2/Bax in hepatic tissue. One study [25] shows that geniposide had a significant effect on increasing the level of caspase-3 in cerebral tissue($P < 0.05$). One study [32] shows geniposide had a significant effect on increasing the ratio of RCR and ST3 in mitochondria($P < 0.01$). One study [27] shows that geniposide had a significant effect on reducing the level of MCP-1 in hepatic tissue ($P < 0.05$). We summarized a schematic representation for the possible intrinsic mechanisms of geniposide protection for organ I/R injury (Fig. 7).

3.6. Identification of mechanisms of IRI amelioration by geniposide

The UniProt database were used to search the gene names of the geniposide targets which were collected by searching the TCMSP database. And 23 geniposide targets were finally chosen after removing duplicate data. Then the term “ischemia reperfusion injury” was searched in a series of disease Target Databases including TTD databases, DisGeNET databases and GeneCards databases and 1373 disease targets which also removed duplications were accessed. By comparing the two sets of data, totally 12 common gene targets were isolated separately and 7 of these were eventually identified as being closely related.

Previous research has shown that the main 7 intersecting targets which were built a PPI network model (Fig. 8) were associated with the following changes: redox status [36,37], inflammation [38] autophagy [39] and apoptosis [40]. The network pharmacology results were symmetrical with the protection mechanisms of the factors which were screened based on studies related to geniposide , and provided another scientific methodological basis for our research.

4. Discussion

Summary of Evidence. According to a rigorous attitude, this study is the initial systematic review including 13 studies with 291 animals to assess the potential beneficial effects of geniposide on I/R injury. The quality of included original studies was generally moderate. The evidence suggests that geniposide appears to have a significant impact on I/R injury through anti-apoptosis, anti-inflammatory, antioxidation, improvement of autophagy and energy metabolism.

Limitations. It still has several limitations we found in the current study and we have summarized these inadequacies as follows. Firstly, we collected relevant articles only in English and Chinese. Thus, there could be some potentially valuable studies in other languages which were ignored. Secondly, all models only used male health rats but not combined with hypertension, diabetes, or hyperlipidemia. Meanwhile, agedness was not considered. Thirdly, the number of included studies was limited. Moreover, the included studies existed publication bias for lack of double-blind design and enough sample size. Only one study adopted blinded induction of model [27] while others ignored it. All these points tend to make effect measures imprecise. Nevertheless, the observation that there are limited studies highlights the need for more studies, and the future studies should enlarge the numbers of animals and

adopt blinded induction of model.

Implications. To date, geniposide as a therapeutic for I/R injury on organs has been extensively used to treatment kinds of organic diseases. For cerebral I/R injury, geniposide could decrease the size of cerebral infarction and nerve cell apoptosis rate, increase nerve cell viability, and receive lower neurological scores and behavior scores. The mechanism is enumerated as follows: 1) improving anti-apoptotic functions via elevating GluN2A/Akt/ERK pathways [27] and reducing the level of Caspase-9 and Caspase-3 [35]; 2) inhibiting inflammatory responses via inhibition of the TLR4/MyD88 signaling pathway and its downstream NF-κB and MAPK pathways [24], as well as improving autophagy and the four arachidonic acid pathway [23,28]; 3) anti-oxidation by improving mitochondrial respiratory function [32] and reduction of NO toxicity and NOS activity [29,31]. The present review demonstrates geniposide had significant function on myocardial I/R injury, which can result in lower cardiomyocytes apoptosis rate, better cardiomyocytes cell viability, and reduce MI size. Geniposide played a role in the cardioprotective effect which was achieved by the following mechanisms: 1) decreasing the expression of ATG5, Beclin-1, the ratio of LC3-II/LC3-I and upregulating the expression of p62 and Bcl-2/Bax ratio by activating Akt/mTOR signaling pathways to inhibit autophagy and apoptosis [30]; 2) activating of AMPK signaling, decreasing ROS/TXNIP/NLRP3 signaling pathways which mediating inflammation, and downregulating the mRNA level of NLRP3, N-GSDMD, ASC, caspase-1 to inhibit oxidation and pyroptosis [33]. For hepatic I/R injury, geniposide could significant improved hepatic I/R injury by reducing inflammation response and apoptosis via activation of the PI3K/Akt/mTOR signaling pathway [27], as well as by attenuating the increased levels of cytochrome *c* protein expression, tBid and caspase-3 activity [25]. Besides, geniposide can reduce oxidative stress via increased HO-1 protein expression, which can provide significant hepatoprotection against I/R injury [25]. For renal I/R injury, geneposid inhibited inflammation by the NF-κB pathway and also suppressed oxidative stress [26]. Despite geniposide's potential organ-protection in I/R injury, its therapeutic target hasn't been adequately validated. It is highly scientific that use gene knockout/knockdown *in vivo*, CRISPR/Cas9 *in vitro*, pharmacological inhibitors, and small interfering RNA (SiRNA) to verify the medical target in experimental research. However, in this research, we found only two studies [17,27] that applied pharmacological inhibitors while the others missed. The following research should pay much attention to verifying the geniposide therapeutic target via gene knockout/knockdown *in vivo* or CRISPR/Cas9 *in vitro*, and complement the gaps in previous research, especially in the liver, kidney and so on.

High-quality research methods are the prerequisite for applying the results of animal research to clinical drug treatment of patients [41]. It is argued that harnessing the systematic review is beneficial, which can not only acquire preclinical design and clinical reliability, but also avoid useless experiments, and then develop better experimental animal research [42]. Based on the amended CAMARADES 10-item checklist, much of the included animal studies of this research missed sample size estimation and blinding. Thus, the scores of included studies are generally moderate, due to the absence of the above crucial standards in study design. Considering the smaller sample size of animal experiments than human experiment [43] and the treatment group selection bias, it generally leads to a higher risk of bias. Previous studies have shown that the risk of false-positive results in putative animal models would rise when the experiment is not designed including blinding [43]. Therefore, it may be easier to demonstrate the efficacy of geniposide in the I/R injury without blinding model induction or outcome assessment. Moreover, animal models as an effective method can better enhance our mechanisms understanding and clinical diseases etiology [44]. Our research finds that searching all the included articles, only two studies used the mice [25,26], while others chose rats. But rodents still have some weaknesses, for example, they are not very similar to human beings and have a short survival time. Furthermore, in clinical, ischemia diseases with multifactorial risk factors such as hypertension and dyslipidaemia are quite common [45]. But none of the 13 included animal studies involved advanced age, hypertension, diabete and hyperlipidemia. The ways of the improvement of I/R injury animal studies are as follows: 1) adding the blinding and sample size estimation in the experiment design course. 2) choosing appropriate animal models more closely related to human anatomy and pharmacodynamics. 3) enriching the kinds of animal models such as ischemia with hypertension or dyslipidaemia which can concord with real clinical patients.

Although organs that are in an ischemic situation have irreversible tissue injury, reperfusion perse would contribute to severe inflammatory responses no matter in local or systemic, over-express of which could lead to serious tissue injury. What's more, if lacking timely treatment, I/R injury can result in multiple organ failure or fatality [46]. Unfortunately, despite researchers have gained a deeper understanding of I/R injury mechanism, the effective treatment of I/R injury is still confused. To date, a series of preclinical trials utilizing geniposide preparations have revealed an outstanding effect of geniposide in the treatment of I/R injury, including cerebral, myocardial, hepatic, and renal I/R injury. In addition, recent studies demonstrate that genipin, a metabolite of geniposide, could reduce cerebral I/R injury via inhibiting the UCP2-SIRT3 signaling pathway and improve hepatic I/R injury [47–49]. And thus, We should test whether metabolites of geniposide are more effective than geniposide itself. This review of I/R injury evaluated the efficacy of geniposide alone, in fact, many metabolites of *Gardenia jasminoides* Ellis such as genipin can be summarized as potential candidates for anti-I/R injury drugs. Further research should test these metabolites directly, and then explore their efficacy, safety and toxicity. It will be a novel research strategy for geniposide as a therapeutic for I/R injury.

5. Conclusion

According to this systematic review, geniposide may be a potential therapeutic intervention in I/R injury. In this review, we explored molecular mechanisms of geniposide in I/R injury. Through meta-analysis and network pharmacology of present study, it is pronounced that geniposide has a protective effect in I/R injury by inhibiting apoptosis, oxidation, inflammation and improving autophagy and mitochondrial respiration. Despite a small portion of research methods may reduce the reliability of positive results, this preclinical systematic review, which was the first one summarizing the relationship between geniposide and ischemia diseases, still shows a novel therapy for I/R injury and appears an enticing implication of geniposide in I/R injury.

Author contribution statement

Chaoqin Luo, Lingfeng Wang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Yifan Wu, Menghan Liu, Yuqiao Lu: Contributed reagents, materials, analysis tools or data.
Baoxin Chen, Yunling Zhang: Conceived and designed the experiments.
Chen Fu: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Xuemei Liu: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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