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**Effect of dl-3-n-butylphthalide on infarction volume in animal models of ischemic stroke: A meta-analysis**

Luan D *et al.* Dl-3-n-butylphthalide reduces cerebral infarction volume

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**Abstract**

***BACKGROUND***

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality. Dl-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy.

***AIM***

To evaluate the effect of NBP on infarct volume in experimental ischemic stroke.

***METHODS***

Twenty-one relevant articles were included from the PubMed, EMBASE, Web of Science, Chinese National Knowledge Infrastructure, VIP information database, and Wanfang database, and data on the effect of dl-3-n-butylphthalide on infarction volume in the middle cerebral artery occlusion models were extracted. Statistical analyses of standard mean difference (SMD) were performed using a random effects model with Revman 5.3.

***RESULTS***

Meta-analysis of the 21 studies suggested that NBP significantly reduced the cerebral infarction volume in middle cerebral artery occlusion model animals compared to the control group [SMD = -3.97, 95%CI: -4.71 to -3.23, *P* < 0.01; heterogeneity: χ2 = 59.09, df = 20 (*P* < 0.01); *I*2 = 66 %].

***CONCLUSION***

NBP is effective in the treatment of experimental ischemic stroke.

**Key words:** Butylphthalide; Animal model; Ischemic stroke; Meta-analysis

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**Core tip:** The systematic review of animal research is of great significance in drug development. This study reports for the first time a systematic review and meta-analysis of the effects of butylphthalide on the volume of cerebral infarction in experimental ischemic stroke.

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**INTRODUCTION**

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality[1,2]. The current treatment includes drug-based thrombolysis and interventional therapy in acute stage, however, there are many inherent limitations in them[3,4]. To date, more than 1000 clinical trials of potential neuroprotective drugs have been verified to fail[5].

Dl-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy; the injectable formulations have been approved for the treatment of acute ischemic stroke in China[6]. NBP protects the integrity of cerebrovascular structures[7], promotes the formation of collateral circulation, accelerates the proliferation of neonatal capillary[8,9], and increases the cerebral blood perfusion[10]; by targeting mitochondria, it improves neuronal energy metabolism[11] and reduces oxidative stress damage and neuronal apoptosis[12]. The systematic review of animal research is of great significance in drug development[13]. To this end, we conducted a meta-analysis of preclinical studies to evaluate the efficacy and mechanisms of NBP in experimental ischemic stroke.

**MATERIALS AND METHODS**

***Literature search strategies***

This meta-analysis was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[14]. All Chinese and English articles published before August 2018 regarding the effects of NBP on experimental ischemic stroke were searched in the six databases, which included PubMed, EMBASE, Web of Science, Wanfang database, VIP Chinese Journal Service Platform database, and China National Knowledge Infrastructure database. Furthermore, to further retrieve the relevant literature, we searched the list of references for potential publications. In the search of the Web of Science, PubMed, and EMBASE databases, only one keyword of “butylphthalide” was used. In the search of other databases, the following search strategy was utilized: “butylphthalide” AND “cerebral ischemia OR brain ischemia OR cerebral infarction OR brain infarction OR stroke OR cerebral ischemia/reperfusion OR cerebral I/R”.

***Inclusion and exclusion criteria***

The inclusion criteria were: (1) the experimental ischemic stroke model was established by the adoption of middle cerebral artery occlusion (MCAO); (2) the intervention group used NBP and the control group applied blank or non-functional solvent; and (3) the cerebral infarct volume was included in the study results, the unit of infarct volume was “%”, and the calculation formula was (infarction volume / whole brain volume) × 100%. The exclusion criteria were: (1) the intervention group was not administered with NBP or given NBP with other medicines concomitantly; (2) MCAO was not adopted for developing the animal models; (3) no control group was included; (4) repeating literature; and (5) the data were not available.

***Literature screening and data extraction***

By reading the title, abstract, and full text according to the inclusion and exclusion criteria, the literature and extracted data were screened independently and cross-checked by the first author and the second author. When there was a disagreement, the point must be reached through the discussion panel which consisted of all authors, and the final conclusion was determined by the corresponding author.

The following data were extracted from the included literature: (1) the year of publication and the name of the first author; (2) the species, age, weight, gender, anesthesia methods, and model types of experimental animals (transient MCAO or permanent MCAO); (3) therapeutic dose, route of administration, time of onset of treatment, and duration of interventional treatment; (4) the mean value and standard deviation of the cerebral infarction volume; and (5) the potential therapeutic mechanism of NBP for ischemic stroke.

Moreover, in case of the use of multiple doses and multiple time points across studies, the final experimental data using the highest dose were extracted. If the volume of cerebral infarction cannot be obtained directly from the original text, the author of the literature was contacted by e-mail to get complete data, and if not, calculation was performed by using digital scale software.

***Quality assessment***

The risk of bias tool of the Systematic Review Centre for Laboratory animal Experimentation’s was applied to assess the methodological quality of the included studies[15]: (1) baseline characteristics: the strain, gender, age, weight, anesthesia methods, name, and anesthetic dose of the experimental animals were involved; (2) allocation concealment: the experimental animals were grouped randomly; (3) sequence generation: the generation of allocation sequence was random; (4) random housing: the living environment and feeding conditions of each group of animals were in conformity with those of each group; (5) blindly feeding: the blind method was adopted for the breeder; (6) random outcome assessment: the outcomes were evaluated randomly on the premise of random selection of animals; (7) result evaluator blindness: the blind method was adopted by the outcome evaluator; (8) complete outcome data: all data of animals were included in the final analysis; (9) no selective outcome reporting: no report bias; and (10) no other sources of bias: there were no other factors that contributed to the risk of high bias.

***Statistical analysis***

Revman 5.3 software was used to analyze all data. Since the cerebral infarction volume was considered as continuous data, the standard mean difference was used to assess the combined effect sizes using a random effects model.

**RESULTS**

***Study inclusion***

A total of 5149 relevant articles were retrieved from six databases, among which 4630 duplicate or irrelevant articles were excluded, resulting in 519 articles for further evaluation. After scanning the titles and abstracts of the remaining articles, 449 were rejected for being review comments, reviews, case reports, clinical trials, and editorials. By reading the full text of the remaining 70 articles, 49 were eliminated as the animal model was not established by the MCAO method, the intervention drug was not NBP monotherapy, there was no control group, or the infarct volume calculation formula did not meet the inclusion criteria. Ultimately, 21 eligible articles were identified[8,16-35] (Figure 1).

***Study characteristics***

A total of 21 articles were collected, among which 5[24,25,32,33,35] were published in Chinese and the remaining were in English. A total of 314 animals, including 159 in the experimental group and 155 in the control group, were involved in 21 studies to investigate the effect of NBP on the volume of cerebral infarction in the experimental ischemic stroke model. Moreover, in 21 articles, there were 15 studies performed on SD rats (15/21, 71.4%), 3[25,29,35] on Wistar rats (3/21, 14.3%), 1[22] on C57BL6/J mice (1/21, 4.8%), 1[16] on CD1 mice (1/21, 4.8%), and 1[31] on 129S2/Sv mice (1/21, 4.7%); 21 studies used male animals (20/21, 95.2%), and one used both male and female animals (1/21, 4.8%)[18]. As for anesthesia method, intraperitoneal injection of chloral hydrate was used in 14 (14/21, 66.7%) studies, injection of sodium pentobarbital into the abdominal cavity in 1(1/21, 4.8%)[19], ketamine and xylazine injection into the peritoneal cavity in 1(1/21, 4.7%)[29], and isoflurane inhalation in 1(1/21, 4.8%)[22]. Only one study[34] adopted both atropine sulfate to reduce airway secretions and inhalation anesthesia with isoflurane (1/21, 4.7%). In addition, 3 studies[17,18,33] did not mention the anesthesia methods (3/21, 14.3%). The animal models used in 15 studies were transient MCAO (tMCAO) (15/21, 71.4%), and a permanent MCAO (pMCAO) was adopted in 6 (6/21, 28.6%) studies[16,25,29,33,35]. To detect infarction volume, 19 and 2[8,22] studies used 2,3,5-triphenyltetrazolium chloride and cresyl violet as the staining agents, respectively (Table 1).

***Study quality***

Of the 21 studies, five got 7 points, four got 6, eight got 5, two got 4, and two got 3. None of the studies described blind feeding and random outcome assessment; the result evaluator blindness was described only in 2 studies[8,32]; all studies described the data of baseline characteristics; 2 studies[18,29] found other sources of bias; no incomplete outcome data, and no selective outcome reporting were described in 11 and 17 studies, respectively (Table 2).

***Effectiveness***

Meta-analysis of the 21 studies suggested that NBP significantly reduced the cerebral infarction volume of MCAO model animals compared to the control group [SMD = -3.97, 95%CI: -4.71 to -3.23, *P* < 0.01; heterogeneity: *χ*2 = 59.09, df = 20 (*P* < 0.01); *I*2 = 66 %] (Figure 2). Moreover, meta-analysis of 15 studies adopting the tMCAO model also verified that NBP significantly reduced infarct volume [SMD = -3.67, 95%CI: -4.52 to -2.82, *P* < 0.01; heterogeneity: χ2 = 42.34, df = 14 (*P* < 0.01); *I*2 = 67%] (Figure 3A). The same is true for studies using the pMCAO model [SMD = -4.70, 95%CI: -5.92 to -3.47, *P* < 0.01; heterogeneity: χ2 = 9.26, df = 5 (*P* = 0.10); *I*2 = 46%] (Figure 3B). When analyzing the effects of pre- or post-administrated NBP on the volume of cerebral infarction in the MCAO model, it was found that both pre-administration [SMD = -3.93, 95%CI: -5.51 to -2.36, *P* < 0.01; heterogeneity: χ2 = 25.58, df= 6 (*P* < 0.01); *I*2 = 77%] (Figure 4A) and post-administration [SMD = -3.62, 95%CI: -4.32 to -2.92, *P* < 0.01; heterogeneity: χ2 = 17.92, df = 11 (*P* =0.08); *I*2 = 39%] (Figure 4B) reduced the infarct volume of the model animals. A funnel plot was adopted to evaluate publication bias and slight bias was found (Figure 5A).

**DISCUSSION**

***Summary of evidence***

The preclinical meta-analysis study evaluated the effects of NBP on infarct volume in experimental ischemic stroke, which was based on experimental data from 314 animals in five Chinese articles and sixteen English articles. The evidence obtained from the present study suggests that NBP might play potential neuroprotective roles for ischemic stroke by increasing cerebral blood flow, enhancing mitochondrial function, protecting integrity of the structure and function of blood-brain barrier, and exerting anti-inflammatory and antioxidant stress activities.

***Limitations***

First, the absence of the relevant literature in other languages other than Chinese and English may lead to selective bias. Second, none of studies provided sample size calculations, blind feeding, and random outcome assessment. Third, the lack of negative research may result in an overestimation of the efficacy of NBP. Fourth, cerebral infarction is usually accompanied by other conditions, such as old age, hypertension, hyperlipidemia, diabetes, and heart disease[36-39]. We did not analyze the effects of NBP on cerebral infarction when the accompanying situation occurred. Fifth, one study[18] using female animals did not rule out estrogen neuroprotection, which has been reported[40]. One study[29] of anesthetic drugs containing ketamine did not eliminate its neuroprotection, which has been reported in preclinical and clinical studies[41,42].

***Potential neuroprotective mechanisms***

Summarizing the included literature, we found that NBP plays a neuroprotective role in experimental ischemic stroke by acting on multiple targets (Figure 5B). We drew a conclusion of the underlying mechanisms as follows: (1) Increasing blood supply to brain tissue in the ischemic area by dilating the middle cerebral artery[8], regulating the expression of *REN, AGT, ACE 1, AGTR 1, RoA, PTGIS, PTGES,* and *TBXAS 1* in ischemic brain tissue[8], decreasing TXB2 and 6-keto-PGF1α ratio[27], and reducing thrombosis; (2) Promoting angiogenesis by increasing VEGF and bFGF in ischemic brain tissue[16,26,32,33]; (3) Exerting anti-inflammatory activity by inhibiting the TLR4/NF-κB signaling pathway[19,21] and decreasing the expression of S100B in ischemic brain tissue[25]; (4) Protecting the structure and function of the blood-brain barrier by modulating the expression of *MMP-9* and *claudin-5* in ischemic brain tissue[16,22], upregulating the expression of *GFAP* in ischemic brain tissue, stabilizing astrocytes[16], increasing the expression of TIMP1, and decreasing the expression of SBP in ischemic brain tissue[22]; (5) Exerting antioxidative stress activity by enhancing the Nrf-2/HO-1 signaling pathway[16], reducing the expression of ROS and MDA in ischemic brain tissue, and increasing the expression of SOD, GSH-px, GSH, Trx, and Txnip in ischemic brain tissue[17,20,21,28]; (6) Protecting the structure and function of mitochondria by increasing the expression of Bcl-2 in ischemic brain tissue[21], reducing the expression of Smac in ischemic brain tissue[25], and reducing mitochondrial release of cytochrome C and AIF[31]; and (7) Exerting anti-apoptosis activity by reducing the expression of cleaved caspase-3, p-p38, and p-JNK in ischemic brain tissue[17,31], increasing the expression of HGF and p-ERK in ischemic brain tissue[19,22], decreasing the expression of GRP78 and CHOP in ischemic brain tissue, and inhibiting endoplasmic reticulum stress-induced apoptosis[24].

***Implications***

Animal experiments are an important link between basic research and clinical experiments. The results have reference value for the next step in designing and implementing clinical research. Compared with clinical research, the principles of randomization and blindness are theoretically easier to be implemented in animal experiments. Animal research is important for comprehending disease mechanisms, and high-quality preclinical research is also critical for translational medicine[43,44]. Therefore, to obtain more accurate and less biased experimental data, designing animal programs should follow the guidelines all the time[15,45], calculate sample size in the beginning, apply applicable animals, use appropriate anesthetic drugs, adopt random feeding, blind models during the experiment, and employ random outcome measurements at the time of evaluation.

Similar to artemisinin, NBP is also a plant-derived drug approved for the treatment of acute ischemic stroke in China. We envision that NBP may be used to treat more patients in the world, like artemisinin, and it requires a large number of randomized, double-blind, and multi-center clinical trials in terms of safety and efficacy.

In conclusion**,** we have conducted the first preclinical systematic review and meta-analysis of the effects of NBP on experimental ischemic stroke, and found that NBP is effective in the treatment of experimental ischemic stroke.

**ARTICLE HIGHLIGHTS**

***Research background***

Ischemic stroke is a frequently-occurring disease in the elderly and characterized by high morbidity and mortality. Dl-3-n-butylphthalide (NBP), a synthetic compound based on natural celery seeds, has potential therapeutic effects on cerebral ischemia, brain trauma, memory impairment, and epilepsy. The systematic review of animal research is of great significance in drug development.

***Research motivation***

There are many studies on the therapeutic effects of NBP in the middle cerebral artery occlusion model, and there is controversy about whether NBP reduces the volume of cerebral infarction.

***Research objectives***

To evaluate the effect of NBP on infarct volume in experimental ischemic stroke.

***Research methods***

We searched Chinese and English databases to screen NBP-related literature. Data such as cerebral infarction volume and potential therapeutic mechanisms were extracted. The risk of bias tool of the Systematic Review Centre for Laboratory animal Experimentation’s was applied to assess the methodological quality of the included studies. Data analysis was performed using Revman 5.3 software.

***Research results***

Meta-analysis of the 21 studies had suggested that NBP significantly reduced the cerebral infarction volume of middle cerebral artery occlusion (MCAO) model animals compared to the control group. Moreover, meta-analysis of 15 studies adopting the tMCAO model also verified that NBP reduced infarct volume significantly. The same is true for studies using the pMCAO model. When analyzing the effects of pre- or post-administrated NBP on the volume of cerebral infarction in the MCAO model, it was found that both pre-administration and the post-administration reduced the infarct volume of the model animals.

***Research conclusions***

NBP is effective in the treatment of experimental ischemic stroke.

***Research perspectives***

Animal experiments are an important link between basic research and clinical experiments. The results have reference value for the next step in designing and implementing clinical research. Compared with clinical research, the principles of randomization and blindness are theoretically easier to be implemented in animal experiments. Animal research is important for comprehending disease mechanisms, and high-quality preclinical research is also critical for translational medicine. Therefore, to obtain more accurate and less biased experimental data, designing animal programs should follow the guidelines all the time, calculate sample size in the beginning, apply applicable animals, use appropriate anesthetic drugs, adopt random feeding, blind models during the experiment, and employ random outcome measurements at the time of evaluation.

**REFERENCES**

1 **Feigin VL**, Norrving B, Mensah GA. Global Burden of Stroke. *Circ Res* 2017; **120**: 439-448 [PMID: 28154096 DOI: 10.1161/CIRCRESAHA.116.308413]

2 **Writing Group Members**, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation* 2016; **133**: e38-360 [PMID: 26673558 DOI: 10.1161/CIR.0000000000000350]

3 **de Ridder IR**, Fransen PS, Beumer D, Berkhemer OA, van den Berg LA, Wermer MJ, Lingsma H, van Zwam WH, Roos YB, van Oostenbrugge RJ, Majoie CB, van der Lugt A, Dippel DW. Is Intra-Arterial Treatment for Acute Ischemic Stroke Less Effective in Women than in Men? *Interv Neurol* 2016; **5**: 174-178 [PMID: 27781046 DOI: 10.1159/000447331]

4 **Chen HS**, Qi SH, Shen JG. One-Compound-Multi-Target: Combination Prospect of Natural Compounds with Thrombolytic Therapy in Acute Ischemic Stroke. *Curr Neuropharmacol* 2017; **15**: 134-156 [PMID: 27334020]

5 **Jickling GC**, Sharp FR. Improving the translation of animal ischemic stroke studies to humans. *Metab Brain Dis* 2015; **30**: 461-467 [PMID: 24526567 DOI: 10.1007/s11011-014-9499-2]

6 **Wang S**, Ma F, Huang L, Zhang Y, Peng Y, Xing C, Feng Y, Wang X, Peng Y. Dl-3-n-Butylphthalide (NBP): A Promising Therapeutic Agent for Ischemic Stroke. *CNS Neurol Disord Drug Targets* 2018; **17**: 338-347 [PMID: 29895257 DOI: 10.2174/1871527317666180612125843]

7 **Zhao Y**, Li J, Zhang P, Chen C, Li S. Protective effects of dl-3n-butylphthalide against diffuse brain injury. *Neural Regen Res* 2013; **8**: 2615-2624 [PMID: 25206572 DOI: 10.3969/j.issn.1673-5374.2013.28.003]

8 **Qin C**, Zhou P, Wang L, Mamtilahun M, Li W, Zhang Z, Yang GY, Wang Y. Dl-3-N-butylphthalide attenuates ischemic reperfusion injury by improving the function of cerebral artery and circulation. *J Cereb Blood Flow Metab* 2018: 271678X18776833 [PMID: 29762050 DOI: 10.1177/0271678X18776833]

9 **Zhao Y**, Lee JH, Chen D, Gu X, Caslin A, Li J, Yu SP, Wei L. DL-3-n-butylphthalide induced neuroprotection, regenerative repair, functional recovery and psychological benefits following traumatic brain injury in mice. *Neurochem Int* 2017; **111**: 82-92 [PMID: 28359729 DOI: 10.1016/j.neuint.2017.03.017]

10 **Xiong Z**, Lu W, Zhu L, Zeng L, Shi C, Jing Z, Xiang Y, Li W, Tsang CK, Ruan Y, Huang L. Dl-3-n-Butylphthalide Treatment Enhances Hemodynamics and Ameliorates Memory Deficits in Rats with Chronic Cerebral Hypoperfusion. *Front Aging Neurosci* 2017; **9**: 238 [PMID: 28798681 DOI: 10.3389/fnagi.2017.00238]

11 **Liu RZ**, Fan CX, Zhang ZL, Zhao X, Sun Y, Liu HH, Nie ZX, Pu XP. Effects of Dl-3-n-butylphthalide on Cerebral Ischemia Infarction in Rat Model by Mass Spectrometry Imaging. *Int J Mol Sci* 2017; **18**: E2451 [PMID: 29165327 DOI: 10.3390/ijms18112451]

12 **Feng L**, Sharma A, Niu F, Huang Y, Lafuente JV, Muresanu DF, Ozkizilcik A, Tian ZR, Sharma HS. TiO2-Nanowired Delivery of DL-3-n-butylphthalide (DL-NBP) Attenuates Blood-Brain Barrier Disruption, Brain Edema Formation, and Neuronal Damages Following Concussive Head Injury. *Mol Neurobiol* 2018; **55**: 350-358 [PMID: 28856586 DOI: 10.1007/s12035-017-0746-5]

13 **de Vries RB**, Wever KE, Avey MT, Stephens ML, Sena ES, Leenaars M. The usefulness of systematic reviews of animal experiments for the design of preclinical and clinical studies. *ILAR J* 2014; **55**: 427-437 [PMID: 25541545 DOI: 10.1093/ilar/ilu043]

14 **Stewart LA**, Clarke M, Rovers M, Riley RD, Simmonds M, Stewart G, Tierney JF; PRISMA-IPD Development Group. Preferred Reporting Items for Systematic Review and Meta-Analyses of individual participant data: the PRISMA-IPD Statement. *JAMA* 2015; **313**: 1657-1665 [PMID: 25919529 DOI: 10.1001/jama.2015.3656]

15 **Hooijmans CR**, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; **14**: 43 [PMID: 24667063 DOI: 10.1186/1471-2288-14-43]

16 **Zhao YJ**, Nai Y, Ma QS, Song DJ, Ma YB, Zhang LH, Mi LX. Dl-3-n-butylphthalide protects the blood brain barrier of cerebral infarction by activating the Nrf-2/HO-1 signaling pathway in mice. *Eur Rev Med Pharmacol Sci* 2018; **22**: 2109-2118 [PMID: 29687870 DOI: 10.26355/eurrev\_201804\_14744]

17 **Yan RY**, Wang SJ, Yao GT, Liu ZG, Xiao N. The protective effect and its mechanism of 3-n-butylphthalide pretreatment on cerebral ischemia reperfusion injury in rats. *Eur Rev Med Pharmacol Sci* 2017; **21**: 5275-5282 [PMID: 29228445 DOI: 10.26355/eurrev\_201711\_13852]

18 **Wang Y**, Huang Y, Xu Y, Ruan W, Wang H, Zhang Y, Saavedra JM, Zhang L, Huang Z, Pang T. A Dual AMPK/Nrf2 Activator Reduces Brain Inflammation After Stroke by Enhancing Microglia M2 Polarization. *Antioxid Redox Signal* 2018; **28**: 141-163 [PMID: 28747068 DOI: 10.1089/ars.2017.7003]

19 **Zhang P**, Guo ZF, Xu YM, Li YS, Song JG. N-Butylphthalide (NBP) ameliorated cerebral ischemia reperfusion-induced brain injury via HGF-regulated TLR4/NF-κB signaling pathway. *Biomed Pharmacother* 2016; **83**: 658-666 [PMID: 27468961 DOI: 10.1016/j.biopha.2016.07.040]

20 **Yin W**, Lan L, Huang Z, Ji J, Fang J, Wang X, Ji H, Peng S, Xu J, Zhang Y. Discovery of a ring-opened derivative of 3-n-butylphthalide bearing NO/H2S-donating moieties as a potential anti-ischemic stroke agent. *Eur J Med Chem* 2016; **115**: 369-380 [PMID: 27031213 DOI: 10.1016/j.ejmech.2016.03.044]

21 **Hua K**, Sheng X, Li TT, Wang LN, Zhang YH, Huang ZJ, Ji H. The edaravone and 3-n-butylphthalide ring-opening derivative 10b effectively attenuates cerebral ischemia injury in rats. *Acta Pharmacol Sin* 2015; **36**: 917-927 [PMID: 26073328 DOI: 10.1038/aps.2015.31]

22 **Lu YM**, Huang JY, Wang H, Lou XF, Liao MH, Hong LJ, Tao RR, Ahmed MM, Shan CL, Wang XL, Fukunaga K, Du YZ, Han F. Targeted therapy of brain ischaemia using Fas ligand antibody conjugated PEG-lipid nanoparticles. *Biomaterials* 2014; **35**: 530-537 [PMID: 24120040 DOI: 10.1016/j.biomaterials.2013.09.093]

23 **Wang X**, Wang L, Li T, Huang Z, Lai Y, Ji H, Wan X, Xu J, Tian J, Zhang Y. Novel hybrids of optically active ring-opened 3-n-butylphthalide derivative and isosorbide as potential anti-ischemic stroke agents. *J Med Chem* 2013; **56**: 3078-3089 [PMID: 23509954 DOI: 10.1021/jm4001693]

24 **Wang W,** Kong W, Chen M, Zheng X, Zhao S, Kong X. Effects of 3-n-butylphthalide pretreatment on endoplasmic reticulum stress in rats with cerebral ischemia reperfusion. *Linchuang Shenjingbingxue Zazhi* 2013; **26**: 122-124

25 **Pan J,** Chen X, Li Q, Song D. Brain protective effect of butylpthalide preconditioning in rat with cerebral ischemics. *Linchuang Shenjingbingxue Zazhi* 2013; **26**: 44-46

26 **Zhang PL**, Lu HT, Zhao JG, Li MH. Protective effect of dl-3n-butylphthalide preconditioning on focal cerebral ischaemia-reperfusion injury in rats. *Acta Neuropsychiatr* 2013; **25**: 12-17 [PMID: 26953069 DOI: 10.1111/j.1601-5215.2012.00649.x]

27 **Wang X**, Zhao Q, Wang X, Li T, Lai Y, Peng S, Ji H, Xu J, Zhang Y. Studies on the enantiomers of ZJM-289: synthesis and biological evaluation of antiplatelet, antithrombotic and neuroprotective activities. *Org Biomol Chem* 2012; **10**: 9030-9040 [PMID: 23076046 DOI: 10.1039/c2ob26511g]

28 **Wu J**, Ling J, Wang X, Li T, Liu J, Lai Y, Ji H, Peng S, Tian J, Zhang Y. Discovery of a potential anti-ischemic stroke agent: 3-pentylbenzo[c]thiophen-1(3H)-one. *J Med Chem* 2012; **55**: 7173-7181 [PMID: 22827516 DOI: 10.1021/jm300681r]

29 **Zhang L**, Yu WH, Wang YX, Wang C, Zhao F, Qi W, Chan WM, Huang Y, Wai MS, Dong J, Yew DT. DL-3-n-Butylphthalide, an anti-oxidant agent, prevents neurological deficits and cerebral injury following stroke per functional analysis, magnetic resonance imaging and histological assessment. *Curr Neurovasc Res* 2012; **9**: 167-175 [PMID: 22621233]

30 **Zhao Q**, Zhang C, Wang X, Chen L, Ji H, Zhang Y. (S)-ZJM-289, a nitric oxide-releasing derivative of 3-n-butylphthalide, protects against ischemic neuronal injury by attenuating mitochondrial dysfunction and associated cell death. *Neurochem Int* 2012; **60**: 134-144 [PMID: 22142531 DOI: 10.1016/j.neuint.2011.11.013]

31 **Li J**, Li Y, Ogle M, Zhou X, Song M, Yu SP, Wei L. DL-3-n-butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway. *Brain Res* 2010; **1359**: 216-226 [PMID: 20800583 DOI: 10.1016/j.brainres]

32 **Cao W,** De Ji Qu Z, Li Q, He L, Zhou D. Effects of dl23n2butylphthalide on the Expression of VEGF and bFGF in Transient Middle Cerebral Artery Occlusion Rats. *Sichuan Daxue Xuebao (Yixue Ban)* 2009; **40**: 403-407

33 **Li Q,** Kong S, De Ji Qu Z, He L, Zhou D. Effects of dI-3-n-butyIphthalide on Expression of VEGF and bFGF in Rat Brain with Permanent Focal Cerebral lschemia. *Sichuan Daxue Xuebao (Yixue Ban)* 2008; **39**: 84-88

34 **Zhang Y**, Wang L, Li J, Wang XL. 2-(1-Hydroxypentyl)-benzoate increases cerebral blood flow and reduces infarct volume in rats model of transient focal cerebral ischemia. *J Pharmacol Exp Ther* 2006; **317**: 973-979 [PMID: 16527903 DOI: 10.1124/jpet.105.098517]

35 **Lin JF**, Feng YP. [Effect of dl-3-n-butylphthalide on delayed neuronal damage after focal cerebral ischemia and intrasynaptosomes calcium in rats]. *Yao Xue Xue Bao* 1996; **31**: 166-170 [PMID: 9206264]

36 **Schumacher K**, Kornej J, Shantsila E, Lip GYH. Heart Failure and Stroke. *Curr Heart Fail Rep* 2018; **15**: 287-296 [PMID: 30062623 DOI: 10.1007/s11897-018-0405-9]

37 **Fan YL**, Zhan R, Dong YF, Huang L, Ji XX, Lu P, Liu J, Li P, Cheng XS. Significant interaction of hypertension and homocysteine on neurological severity in first-ever ischemic stroke patients. *J Am Soc Hypertens* 2018; **12**: 534-541 [PMID: 29678422 DOI: 10.1016/j.jash.2018.03.011]

38 **Dandapat S**, Robinson JG. Guidelines for Management of Hyperlipidemia: Implications for Treatment of Patients with Stroke Secondary to Atherosclerotic Disease. *Curr Neurol Neurosci Rep* 2016; **16**: 24 [PMID: 26838351 DOI: 10.1007/s11910-016-0621-1]

39 **Hu WS**, Lin CL. Use of the progression of adapted Diabetes Complications Severity Index to predict acute coronary syndrome, ischemic stroke, and mortality in Asian patients with type 2 diabetes mellitus: A nationwide cohort investigation. *Clin Cardiol* 2018; **41**: 1038-1043 [PMID: 29896758 DOI: 10.1002/clc.22991]

40 **Céspedes Rubio ÁE**, Pérez-Alvarez MJ, Lapuente Chala C, Wandosell F. Sex steroid hormones as neuroprotective elements in ischemia models. *J Endocrinol* 2018; **237**: R65-R81 [PMID: 29654072 DOI: 10.1530/JOE-18-0129]

41 **Zeng ZW**, Zhang YN, Lin WX, Zhang WQ, Luo R. A meta-analysis of pharmacological neuroprotection in noncardiac surgery: focus on statins, lidocaine, ketamine, and magnesium sulfate. *Eur Rev Med Pharmacol Sci* 2018; **22**: 1798-1811 [PMID: 29630129 DOI: 10.26355/eurrev\_201803\_14599]

42 **Bell JD**. In Vogue: Ketamine for Neuroprotection in Acute Neurologic Injury. *Anesth Analg* 2017; **124**: 1237-1243 [PMID: 28079589 DOI: 10.1213/ANE.0000000000001856]

43 **Fluri F**, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. *Drug Des Devel Ther* 2015; **9**: 3445-3454 [PMID: 26170628 DOI: 10.2147/DDDT.S56071]

44 **Hackam DG**, Redelmeier DA. Translation of research evidence from animals to humans. *JAMA* 2006; **296**: 1731-1732 [PMID: 17032985 DOI: 10.1001/jama.296.14.1731]

45 **Kilkenny C**, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *Osteoarthritis Cartilage* 2012; **20**: 256-260 [PMID: 22424462 DOI: 10.1016/j.joca.2012.02.010]

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**Specialty type:** Medicine, research and experimental

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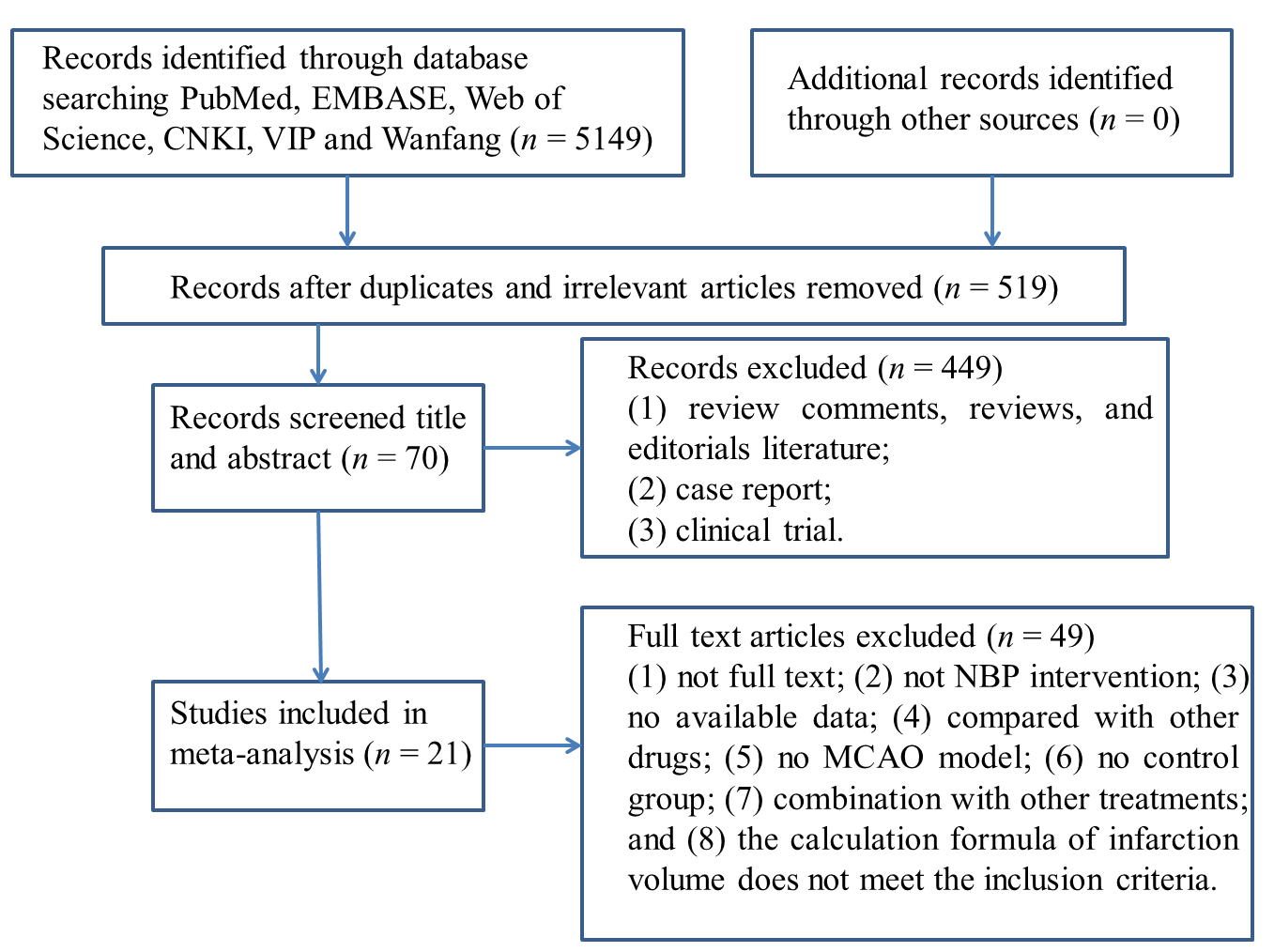
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Grade B (Very good): 0

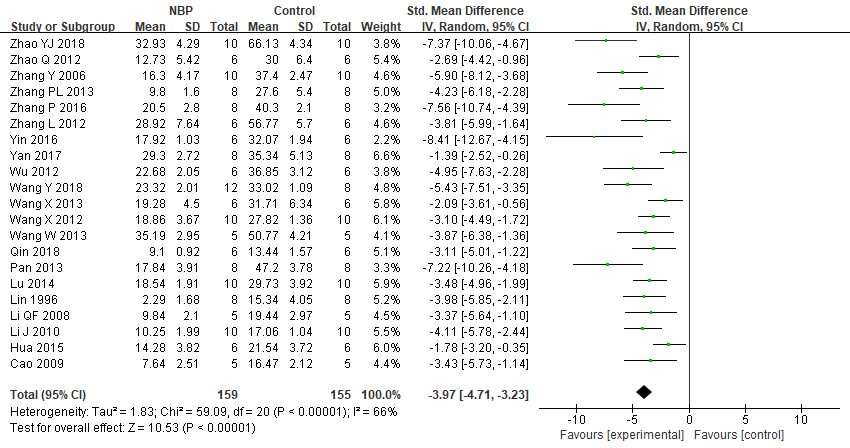
Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0



**Figure 1 Literature inclusion flow chart.** NBP: Dl-3-n-butylphthalide; MCAO: Middle cerebral artery occlusion.



**Figure 2 Forest plot showing effects of dl-3-n-butylphthalide in decreasing cerebral infarction volume compared with the control group.**

E:\我们的征途是星辰大海\butylphthalide\APS投稿用\Fig 3.tif

**Figure 3 Forest plots showing effects of dl-3-n-butylphthalide in decreasing cerebral infarction volume compared with the control group in transient (A) and permanent (B) middle cerebral artery occlusion model, respectively.**

E:\我们的征途是星辰大海\butylphthalide\APS投稿用\Fig 4.tif

**Figure 4 Forest plots showing effects of dl-3-n-butylphthalide in decreasing cerebral infarction volume compared with the control group in pre- (A) and post-administration (B) model, respectively.**

E:\我们的征途是星辰大海\butylphthalide\APS投稿用\Fig 5.tif

**Figure 5 Funnel plot for evaluation of publication bias and underlying mechanism of dl-3-n-butylphthalide in neuroprotection.** A: Funnel plot evaluation of publication bias for the effects of dl-3-n-butylphthalide on infarction volume; B: The underlying mechanism of dl-3-n-butylphthalide in neuroprotection.

**Table 1 Characteristics of the included studies**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study (yr)** | **Species/age**  **Sex/weight**  **Number (control group/experimental group)** | **Anesthetic** | **Model** | **Intervention dose**  **Administration method**  **Time point/duration** | **Measurement of infarction volume** | **Outcome index** | ***P*-value** |
| Qin *et al*[8], 2018 | SD rats/8 weeks | 5 % chloral hydrate | tMCAO 2 h | 90 mg/kg, daily | Cresyl violet | 1 Infarction volume | <0.05 |
|  | Male/250-300 g | 400 mg/kg | Reperfusion 7 d | Gavage | Image J | 2 Ameliorate body weight loss | <0.05 |
|  | 6/6 | Intraperitoneally |  | Postoperative/7 d |  | 3 Improve neurological behavior scores | <0.05 |
|  |  |  |  |  |  | 4 Reduce brain atrophy volume | <0.01 |
|  |  |  |  |  |  | 5 Upregulate *PTGIS* and *PTGES*; downregulate *TBXAS 1* | <0.05 |
|  |  |  |  |  |  | 6 Inhibit *REN*, *AGT*, *ACE 1*, and *AGTR 1*; upregulate *RoA* | <0.05 |
|  |  |  |  |  |  | 7 Increase the diameter of middle cerebral artery | <0.05 |
| Zhao *et al*[16], 2018 | CD 1 mice/10-12 wk | 10% chloral hydrate | pMCAO 24 h | 120 mg/kg | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/27-30 g | 35 mg/g |  |  |  | 2 Improve neurological behavior scores | <0.05 |
|  | 10/10 | Intraperitoneally |  |  |  | 3 Decrease the water content of brain | <0.05 |
|  |  |  |  |  |  | 4 Decrease the permeability of blood-brain barrier | <0.05 |
|  |  |  |  |  |  | 5 Decrease pinocytotic vesicles of capillary endothelial cells | |
|  |  |  |  |  |  | 6 Downregulate *MMP 9* | <0.05 |
|  |  |  |  |  |  | 7 Upregulate *Claudin 5*, *VEGF*, *GFAP*, *Nrf 2*, and *HO 1* | <0.05 |
| Wang *et al*[18], 2018 | SD rats/unknown | Unknown | tMCAO 2 h | 1 mg/kg | 2% TTC | 1 Infarction volume | >0.05 |
|  | Male and female/250-280 g |  | Reperfusion 48 h | Intravenously | Image Pro Plus | 2 Improve neurological behavior scores | >0.05 |
|  | 8/12 |  |  | Postoperative/4 h and 24 h |  |  |  |
| Yan *et al*[17], 2017 | SD rats/adult | Unknown | tMCAO 2 h | 75 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.01 |
|  | Male/180-220 g |  | Reperfusion 24 h | Gavage |  | 2 Decrease the water content of brain | <0.05 |
|  | 8/8 |  |  | Preoperative/7 d |  | 3 Decrease the permeability of blood-brain barrier | <0.01 |
|  |  |  |  |  |  | 4 Decrease cell apoptosis |  |
|  |  |  |  |  |  | 5 Decrease ROS, cleaved caspase-3, and p-p38; increase SOD | <0.01 |
|  |  |  |  |  |  | 6 Decrease MDA and p-JNK | <0.05 |
| Zhang *et al*[19], 2016 | SD rats/unknown | Sodium pentobarbital | tMCAO 1 h | 4.5 mg/kg | TTC | 1 Infarction volume | <0.01 |
|  | Male/250-320 g | 50 mg/kg | Reperfusion 24 h | Intraperitoneally |  | 2 Improve neurological behavior scores | <0.001 |
|  | 8/8 |  |  | Postoperative/- |  | 3 Decrease the water content of brain | <0.05 |
|  |  |  |  |  |  | 4 Upregulate *HGF*; downreglate *TLR4* | <0.001 |
| Yin *et al*[20], 2016 | SD rats/unknown | Chloral hydrate | tMCAO 2 h | 80 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.001 |
|  | Male/280-320 g | 300 mg/kg | Reperfusion 24 h | Gavage | Image Pro Plus | 2 Improve neurological behavior scores | <0.05 |
|  | 6/6 | Intraperitoneally |  | Preoperative/7 d |  | 3 Decrease the water content of brain | <0.01 |
|  |  |  |  |  |  | 4 Decrease MDA | <0.01 |
|  |  |  |  |  |  | 5 Increase SOD | <0.05 |
|  |  |  |  |  |  | 6 Increase GSH-Px | <0.001 |
| Hua *et al*[21], 2015 | SD rats/57-61 days | Chloral hydrate | tMCAO 2 h | 60 mg/kg | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/250-280 g | 300 mg/kg | Reperfusion 24 h | Gavage | Image Pro Plus | 2 Improve neurological behavior scores | >0.05 |
|  | 6/6 | Intraperitoneally |  | Postoperative/- |  | 3 Decrease the water content of brain | <0.01 |
|  |  |  |  |  |  | 4 Decrease MDA; increase GSH, SOD, Nrf 2, Trx, and Bcl-2 | <0.01 |
|  |  |  |  |  |  | 5 Decrease NF-κB p65; increase Txnip | <0.05 |
| Lu *et al*[22],2014 | C57BL6/J/adult | Isoflurane | tMCAO 0.75 h | 10 mg/kg | Cresyl violet | 1 Infarction volume | <0.05 |
|  | Male/unknown | Initiated 3% | Reperfusion 23 h | Intravenous | Image J | 2 Improve neurological behavior scores | <0.05 |
|  | 10/10 | Maintained 1.5% |  | Preoperative/- |  | 3 Decrease MMP 9; increase TIMP 1 | <0.01 |
|  |  |  |  |  |  | 4 Increase SBP and p-ERK | <0.01 |
| Wang *et al*[23], 2013 | SD rats/unknown | Chloral hydrate | tMCAO 2 h | 80 mg/kg, daily | TTC | 1 Infarction volume | <0.05 |
|  | Male/280-320 g | 300 mg/kg | Reperfusion 24 h | Gavage |  | 2 Improve neurological behavior scores | >0.05 |
|  | 6/6 | Intraperitoneally |  | Preoperative/7 d |  | 3 Decrease the water content of brain | >0.05 |
| Wang *et al*[24], 2013 | SD rats/unknown | 10% chloral hydrate | tMCAO 2 h | 80 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/250 ± 20 g (*n* = 30) | 3 mL/kg | Reperfusion 24 h | Gavage |  | 2 Downregulate *GRP78* and *CHOP* | <0.05 |
|  | 5/5 | Intraperitoneally |  | Preoperative/7 d |  |  |  |
| Pan *et al*[25], 2013 | Wistar rats/unknown | 10% chloral hydrate | pMCAO 24 h | 0.8 g/kg, daily | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/200-250 g | 3 mL/kg |  | Gavage | Biosens Digitan Image | 2 Decrease the water content of brain | <0.05 |
|  | 8/8 | Intraperitoneally |  | Preoperative/14 d |  | 3 Decrease Smac and S100B | <0.05 |
| Zhang *et al*[26], 2013 | SD rats/unknown | 6 % chloral hydrate | tMCAO 2 h | 200 mg/kg | 2% TTC | 1 Infarction volume | <0.01 |
|  | Male/250 ± 20 g |  | Reperfusion 24 h | Intraperitoneally | Image Pro Plus | 2 Improve neurological behavior scores | <0.01 |
|  | 8/8 | Intraperitoneally |  | Postoperative/- |  | 3 Increase VEGF | <0.01 |
| Wang *et al*[27], 2012 | SD rats/unknown | Chloral hydrate | tMCAO 2 h | 90 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/250-300 g | 300 mg/kg | Reperfusion 3 d | Gavage |  | 2 Improve neurological behavior scores | <0.05 |
|  | 10/10 | Intraperitoneally |  | Postoperative/3 d |  | 3 Decrease the water content of brain | <0.05 |
|  |  |  |  |  |  | 4 Decrease the ratio of TXB2/6-keto-PGF1α | <0.05 |
| Wu *et al*[28], 2012 | SD rats/unknown | Chloral hydrate | tMCAO 2 h | 80 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.001 |
|  | Male/250-280 g | 350 mg/kg | Reperfusion 24 h | Gavage |  | 2 Improve neurological behavior scores | <0.001 |
|  | 6/6 | Intraperitoneally |  | Preoperative/7 d |  | 3 Decrease the water content of brain | >0.05 |
|  |  |  |  |  |  | 4 Decrease MDA; increase SOD | <0.001 |
| Zhang *et al*[29], 2012 | Wistar Kyoto rats/3 mo | Ketamine, xylazine | pMCAO 7 d | 80 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.01 |
|  | Male/unknown | 75 mg/kg, 10 mg/kg |  | Gavage | Image Pro Plus |  |  |
|  | 6/6 | Intraperitoneally |  | Postoperative/7 d |  |  |  |
| Zhao *et al*[30], 2012 | SD rats/unknown | Chloral hydrate | tMCAO 2 h | 50 mg/kg, daily | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/250-300 g | 300 mg/kg | Reperfusion 3 d | Gavage |  | 2 Improve neurological behavior scores | <0.05 |
|  | 6/6 | Intraperitoneally |  | Postoperative/3 d |  |  |  |
| Li *et al*[31], 2010 | 129S2/Sv/adult | 4 % chloral hydrate | pMCAO 24 h | 100 mg/kg | 2% TTC | 1 Infarction volume | <0.05 |
|  | Male/20-25 g |  |  | Intraperitoneally |  | 2 Decrease cleaved-caspase 3; caspase 9, p-JNK, and p-p38 | <0.05 |
|  | 10/10 | Intraperitoneally |  | Postoperative 1 h/- |  | 3 Reduce mitochondrial release of cytochrome c and AIF | <0.05 |
| Cao *et al*[32], 2009 | SD rats/3-4 mo | 10% chloral hydrate | tMCAO 2 h | 25 mg/kg, twice a day | TTC | 1 Infarction volume | <0.01 |
|  | Male/280-350 g |  | Reperfusion 3 d | Gavage | Image Pro Plus | 2 Improve neurological behavior scores | <0.05 |
|  | 5/5 | Intraperitoneally |  | Postoperative/3 d |  | 3 Upregulate *VEGF* and *bFGF* | <0.05 |
| Li *et al*[33], 2008 | SD rats/3-4 mo | Unknown | pMCAO 3 d | 25 mg/kg, twice a day | TTC | 1 Infarction volume | <0.05 |
|  | Male/280-350 g |  |  | Gavage | Image Pro Plus | 2 Improve neurological behavior scores | <0.05 |
|  | 5/5 |  |  | Postoperative/3 d |  | 3 Upregulate *VEGF* and *bFGF* | <0.05 |
| Zhang *et al*[34], 2006 | SD rats/unknown | 3% isoflurane | tMCAO 2 h | 10 mg/kg | 4% TTC | 1 Infarction volume | <0.001 |
|  | Male/270-330 g | Endotracheal intubation | Reperfusion 24 h | Intravenously | SPOT Biometrics | 2 Improve neurological behavior scores | <0.01 |
|  | 10/10 |  |  | Intraoperative/- |  |  |  |
| Lin *et al*[35], 1996 | Wistar rats/unknown | Chloral hydrate | pMCAO 24 h | 240 mg/kg | 4% TTC | 1 Infarction volume | <0.001 |
|  | Male/250-350 g |  |  | Gavage |  | 2 Improve neurological behavior scores | <0.001 |
|  | 8/8 |  |  | Postoperative/- |  |  |  |

*PTGIS*: Prostacyclin synthase; *PTGES*: Prostaglandin E synthase; *TBXAS 1*: Thromboxane A2 synthase 1; *REN*: Renin; *AGT*: Angiotensinogen; *ACE 1*: Angiotensin converting enzyme 1; *AGTR 1*: Angiotensin II receptor type 1; tMCAO: Transient middle cerebral artery occlusion; pMCAO: Permanent middle cerebral artery occlusion; TTC: 2,3,5-triphenyltetrazolium chloride; *MMP 9*: Matrix metallopeptidase 9; *VEGF*: Vascular endothelial growth factor; *GFAP*: Glial fibrillary acidic protein; *Nrf 2*: NF-E2-related factor 2; *HO 1*: Heme oxygenase 1; ROS: Reactive oxygen species; SOD: Superoxide dismutase; MDA: Malonaldehyde; *HGF*: Hepatocyte growth factor; *TLR4*: Toll like receptor 4; GSH-Px: Glutathione peroxidase; GSH: Glutathione; Trx: Thioredoxin; Txnip: Thioredoxin-interacting protein; TIMP 1: Tissue inhibitor of metalloproteinase 1; SBP: Spectrin breakdown product; *GRP 78*: Glucose-regulated protein 78; CHOP: C/EBP-homologous protein; Smac: Second mitochondria-derived activator of caspases; S100B: S100 calcium binding protein B; TXB2: Thromboxane B2; 6-keto-PGF1α: 6-keto-prostaglandin F1α; AIF: Apoptosis-inducing factor; *bFGF*: Basic fibroblast growth factor.

**Table 2 Risk of bias of the included studies**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study (yr)** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** | **Score** |
| Qin *et al*[8], 2018 | √ | √ | √ |  |  |  | √ | √ | √ | √ | 7 |
| Zhao *et al*[16], 2018 | √ | √ | √ |  |  |  |  |  | √ | √ | 5 |
| Wang *et al*[18], 2018 | √ |  | √ | √ |  |  |  | √ | √ |  | 5 |
| Yan *et al*[17], 2017 | √ |  |  | √ |  |  |  |  | √ | √ | 4 |
| Zhang *et al*[19], 2016 | √ |  |  | √ |  |  |  |  | √ | √ | 4 |
| Yin *et al*[20], 2016 | √ | √ | √ |  |  |  |  | √ | √ | √ | 6 |
| Hua *et al*[21], 2015 | √ | √ | √ | √ |  |  |  | √ | √ | √ | 7 |
| Lu *et al*[22], 2014 | √ | √ | √ | √ |  |  |  | √ | √ | √ | 7 |
| Wang *et al*[23], 2013 | √ | √ | √ |  |  |  |  |  | √ | √ | 5 |
| Wang *et al*[24], 2013 | √ | √ | √ |  |  |  |  | √ |  | √ | 5 |
| Pan *et al*[25], 2013 | √ | √ | √ |  |  |  |  | √ |  | √ | 5 |
| Zhang *et al*[26], 2013 | √ | √ | √ |  |  |  |  |  | √ | √ | 5 |
| Wang *et al*[27], 2012 | √ | √ | √ |  |  |  |  |  | √ | √ | 5 |
| Wu *et al*[28], 2012 | √ | √ | √ |  |  |  |  |  | √ | √ | 5 |
| Zhang *et al*[29], 2012 | √ | √ | √ | √ |  |  |  | √ | √ |  | 6 |
| Zhao *et al*[30], 2012 | √ | √ | √ | √ |  |  |  | √ | √ | √ | 7 |
| Li *et al*[31], 2010 | √ |  |  |  |  |  |  |  | √ | √ | 3 |
| Cao *et al*[32], 2009 | √ | √ | √ | √ |  |  | √ | √ |  | √ | 7 |
| Li *et al*[33], 2008 | √ | √ | √ | √ |  |  |  | √ |  | √ | 6 |
| Zhang *et al*[34], 2006 | √ | √ | √ | √ |  |  |  |  | √ | √ | 6 |
| Lin *et al*[35], 1996 | √ |  |  |  |  |  |  |  | √ | √ | 3 |

A: Baseline characteristics; B: Allocation concealment; C: Sequence generation; D: Random housing; E: Blind feeding; F: Random outcome assessment; G: Result evaluator blindness; H: No incomplete outcome data; I: No selective outcome reporting; J: No other sources of bias.