frontiers | Research Topics

Complementary therapies for neurological disorders: From bench to clinical practices

Edited by
Yang Ye, Jingling Chang, Shuren Li and Lingyong Xiao

Published in
Frontiers in Neurology

ISSN 1664-8714
ISBN 978-2-8325-2865-5
DOI 10.3389/978-2-8325-2865-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Complementary therapies for neurological disorders: From bench to clinical practices

Topic editors

Yang Ye — Peking University Third Hospital, China

Jingling Chang — Beijing University of Chinese Medicine, China

Shuren Li — Medical University of Vienna, Austria

Lingyong Xiao — First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China

Citation

Citation: Ye, Y., Chang, J., Li, S., Xiao, L., eds. (2023). *Complementary therapies for neurological disorders: From bench to clinical practices*.

Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2865-5

Table of contents

frontiers | Frontiers in Neurology

TYPE Editorial
PUBLISHED 14 June 2023
DOI 10.3389/fneur.2023.1224144

OPEN ACCESS

EDITED AND REVIEWED BY
Giorgio Sandrini,
Fondazione Cirna Onlus, Italy

*CORRESPONDENCE
Lingyong Xiao
✉ xlyys@aliyun.com
Jingling Chang
✉ ear6979@163.com

RECEIVED 17 May 2023
ACCEPTED 05 June 2023
PUBLISHED 14 June 2023

CITATION
Ye Y, Li S, Xiao L and Chang J (2023) Editorial: Complementary therapies for neurological disorders: from bench to clinical practices. *Front. Neurol.* 14:1224144. doi: 10.3389/fneur.2023.1224144

Editorial: Complementary therapies for neurological disorders: from bench to clinical practices

Yang Ye[1], Shuren Li[2], Lingyong Xiao[3]* and Jingling Chang[4]*

[1]Department of Traditional Chinese Medicine, Peking University Third Hospital, Beijing, China, [2]Department of Biomedical Imaging and Image-Guided Therapy, Medical University of Vienna, Vienna, Austria, [3]Clinical Department of Acupuncture and Moxibustion, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China, [4]Department of Neurology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China

KEYWORDS

neurological disorder, complementary therapy, stroke, Alzheimer's disease, herb medicine, acupuncture

Editorial on the Research Topic
Complementary therapies for neurological disorders: from bench to clinical practices

Neurological disorders, such as stroke, Parkinson's disease (PD), dementia, and depression, impose heavy burdens on both individuals and society (1). A lot of attempts have been made to ease the symptoms of neurological disorders, but many challenges remain. Complementary therapies, such as acupuncture, moxibustion, herbal medicine, and Tai Chi have been widely used in the treatment of various neurological disorders worldwide, especially in China (2, 3). Numerous clinical trials and animal studies have been conducted to verify the efficacy of complementary therapies and explain their potential mechanisms (4, 5). However, the effectiveness and mechanisms of complementary therapies for treating neurological disorders remain controversial due to insufficient evidence. More well-designed clinical and basic studies in this field are urgently needed.

In order to provide a platform for authors in this field to share their latest research findings, we organized this Research Topic in *Frontiers in Neurology*, section Neurorehabilitation. A total of 53 manuscripts were received during the call for papers, 13 papers among them were finally accepted for publication following a rigorous peer-review process. The 13 accepted articles consist of five original research articles, one brief research report, two reviews, and five systematic review and meta-analyses. Many complementary therapies (e.g., acupuncture, electroacupuncture, and herbal medicine) and neurological disorders (e.g., stroke, depression, and PD) were involved in this Research Topic. These studies have well expanded our current knowledge about complementary therapies for neurological disorders.

Original research article

PD, a progressive neurological disorder characterized by tremor, rigidity, and bradykinesia, is associated with the death of dopaminergic neurons in the brain (6). Jin et al.

performed a pilot clinical trial involving 60 participants to evaluate the efficacy of Ukgansan, a traditional herbal formula composing seven plants, for improving clinical symptoms in patients with PD. This was a single-centered, randomized, controlled, assessor-blinded, pilot clinical trial conducted in South Korea. After 6 weeks of treatment, they found that additional use of Ukgansan improved the quality of life in PD patients with anxiety. Constipation is a common symptom in patients with PD, with a prevalence of 70–80% (7). Song et al. investigated whether electroacupuncture at "Tianshu" (ST25) promotes the restoration of the enteric nervous system and colonic motor function in a rat model of PD constipation. In that study, electroacupuncture at ST25 ameliorated abnormalities of the enteric nervous system and improved constipation symptoms in rats with PD constipation, possibly through maintaining the integrity of the colonic myenteric nervous plexus and regulating the neurotransmitters.

Stroke is a leading cause of death and disability in the world. Hemiplegia after stroke brings a serious burden on the economy and society (8). Lin et al. investigated the functional connectivity changes of cerebral hemispheres, trying to interpret the neural mechanism of scalp acupuncture on hemiplegia after stroke. This clinical study included 21 patients with hemiplegia after stroke and was performed in Beijing, China. They found that scalp acupuncture may promote rehabilitation in patients with hemiplegia after stroke. In addition, scalp acupuncture showed a bidirectional effect including strengthening functional connections of the bilateral motor cortex and weakening abnormal compensatory connections.

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder in children characterized by deficits in social communication, narrow interests, and repetitive behaviors (9). Lee et al. observed the effectiveness and safety of a 6-month integrative treatment program (including herbal medicine, Floortime, and sensory enrichment therapy) on children with ASD in a prospective observational study. This study included 18 participants and was conducted in South Korea. Their results showed that the six-month integrative treatment program significantly relieved the symptoms of ASD children. This treatment program may be a potentially effective therapeutic strategy for ASD, but a placebo control group is needed in the future to further verify the efficacy.

Depression is the most prevalent psychiatric disorder characterized by depressed mood, social isolation, and anhedonia (10). Zhu et al. examined the anti-depressive effects of a traditional Chinese medicine decoction, Zi-Shui-Qing-Gan-Yin (ZSQGY), in a depressive animal model and in a cell model. Their results indicated that ZSQGY effectively improved depressive behavior in depressive animals. The mechanisms may be related to improvement in mitochondrion function, alleviation of neuroinflammation, and regulation of peroxisome proliferator-activated receptor-γ co-activator 1α.

Brief research report

Equistasi® is a vibrotactile device that has been used for rehabilitation in patients with movement disorders such as PD (11). Cruciani et al. briefly reported a pilot study conducted in Italy to explore the effect of Equistasi® on somatosensory processing through the evaluation of high-frequency oscillations. They found that vibrotactile afference delivered by Equistasi® could work through somatosensory processing, rather than by peripheral effects. Equistasi® has the potential to restore equilibrium in disease states such as PD, but the efficacy needs to be further validated and the mechanism needs to be elucidated.

Literature review

Ischemic stroke is a severe neurological disorder with a high mortality and disability rate (12). Acupuncture therapy has been widely used to treat ischemic stroke and the molecular mechanisms involved have been partly elucidated (13). Wang, Su et al. presented a comprehensive review on the mechanisms of acupuncture for enhancing cerebral perfusion in ischemic stroke. They concluded that acupuncture restores blood flow of ischemic tissue possibly via promoting hemodynamics and angiogenesis, releasing vasoactive substances, and improving microcirculation. Acupuncture has shown great potential to improve ischemic stroke in multiple ways and more high-quality clinical trials are needed to verify the efficacy of acupuncture. Cognitive impairment is another important condition that acupuncture has shown therapeutic potential (14). Zhou et al. performed a bibliometric review to explore the development context, research hotspots, and frontiers of acupuncture for cognitive impairment in the past three decades. This review stated that functional magnetic resonance imaging maybe better explain the therapeutic effect of acupuncture. In addition, they found that the effect of acupuncture on a single point is probably more convincing.

Systematic review and meta-analysis

Five systematic review and meta-analyses were published in this Research Topic. Stroke and its complications are still hot topics for researchers. Wang, Chi et al. evaluated the evidence from current systematic reviews of acupuncture for early recovery after acute ischemic stroke. A total of seven systematic reviews including 114 randomized controlled trials (RCTs) were included and assessed in this study. Their results showed that acupuncture is a promising therapy that may improve the neurological function for patients recovering from acute ischemic stroke. However, the low quality of evidence affected the reliability of the results. Shoulder-hand syndrome is a common condition after stroke characterized by pain, hyperalgesia, swelling, and limited joint mobility (15). Feng et al. used Bayesian network meta-analysis to identify the most effective physical therapy for patients with poststroke shoulder-hand syndrome. A total of 45 RCTs were included in the final analysis. According to their results, electromyography biofeedback therapy combined with rehabilitation training is the best physiotherapy option, which could be used for patients with poststroke shoulder-hand syndrome to improve upper extremity motor function and relieve pain. Depression is another common condition affecting about one-third of stroke patients (16). Li et al. performed a systematic review and meta-analysis to assess the efficacy of Chinese herbal medicine (CHM) on poststroke

depression in animal studies. A total of 14 studies with 12 CHMs were included. The results suggested that CHM could significantly improve depression-like behaviors and neurological function of poststroke depression animals.

Dysphagia is a common non-motor symptom in PD. Wu et al. assessed the efficacy of acupuncture on dysphagia in PD patients through meta-analysis method. Ten RCTs with 724 patients were included in this study. Their results showed that acupuncture may exert beneficial effects on dysphagia in PD, which supports acupuncture as an adjunctive treatment for dysphagia in patients with PD.

Diabetic peripheral neuropathy (DPN) is one of the most common complications of diabetes with a high rate of morbidity and mortality (17). Sun et al. systematically evaluated the efficacy of Tongmai Jiangtang Capsule (TJC) on diabetic peripheral neuropathy in patients. It included eight RCTs involving 656 participants for analysis. They found that TJC combined with conventional treatment significantly reduced the severity of DPN symptoms compared with conventional treatment alone. TJC is a promising drug for DPN based on the current evidence, more high-quality evidence is needed to validate the results.

In conclusion, this Research Topic shows the latest research advances both in animal and clinical studies in the complementary therapy field targeting neurological disorders. We believe that these studies will greatly expand our current understanding in this field and help us develop better therapies for patients with neurological disorders.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Acknowledgments

We thank the authors for contributing their valuable work to this Research Topic and the reviewers for their constructive comments. We are also grateful to the editorial board for approving this topic and the editors for their hard work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Guan L, Han Y, Yang C, Lu S, Du J, Li H, et al. CRISPR-Cas9-mediated gene therapy in neurological disorders. *Mol Neurobiol.* (2022) 59:968–82. doi: 10.1007/s12035-021-02638-w

2. Deuel LM, Seeberger LC. Complementary therapies in parkinson disease: a review of acupuncture, Tai Chi, Qi Gong, Yoga, and Cannabis. *Neurotherapeutics.* (2020) 17:1434–55. doi: 10.1007/s13311-020-00900-y

3. Ye Y, Li Q, Pan CS, Yan L, Sun K, Wang XY, et al. QiShenYiQi inhibits tissue plasminogen activator-induced brain edema and hemorrhage after ischemic stroke in mice. *Front Pharmacol.* (2021) 12:759027. doi: 10.3389/fphar.2021.759027

4. Zhao J, Chau JPC, Chan AWK, Meng Q, Choi KC, Xiang X, et al. Tailored sitting Tai Chi program for subacute stroke survivors: a randomized controlled trial. *Stroke.* (2022) 53:2192–203. doi: 10.1161/STROKEAHA.121.036578

5. Yao L, Ye Q, Liu Y, Yao S, Yuan S, Xu Q, et al. Electroacupuncture improves swallowing function in a post-stroke dysphagia mouse model by activating the motor cortex inputs to the nucleus tractus solitarii through the parabrachial nuclei. *Nat Commun.* (2023) 14:810. doi: 10.1038/s41467-023-36448-6

6. Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet.* (2021) 397:2284–303. doi: 10.1016/S0140-6736(21)00218-X

7. Kuai XY, Yao XH, Xu LJ, Zhou YQ, Zhang LP, Liu Y, et al. Evaluation of fecal microbiota transplantation in Parkinson's disease patients with constipation. *Microb Cell Fact.* (2021) 20:98. doi: 10.1186/s12934-021-01589-0

8. Zhang J, Mu Y, Zhang Y. Effects of acupuncture and rehabilitation training on limb movement and living ability of patients with hemiplegia after stroke. *Behav Neurol.* (2022) 2022:2032093. doi: 10.1155/2022/2032093

9. Hirota T, King BH. Autism spectrum disorder: a review. *JAMA.* (2023) 329:157–68. doi: 10.1001/jama.2022.23661

10. Dudek KA, Dion-Albert L, Kaufmann FN, Tuck E, Lebel M, Menard C. Neurobiology of resilience in depression: immune and vascular insights from human and animal studies. *Eur J Neurosci.* (2021) 53:183–221. doi: 10.1111/ejn.14547

11. Spolaor F, Romanato M, Annamaria G, Peppe A, Bakdounes L, To DK, et al. Relationship between muscular activity and postural control changes after proprioceptive focal stimulation (Equistasi((R))) in middle-moderate parkinson's disease patients: an explorative study. *Sensors (Basel).* (2021) 21:560. doi: 10.3390/s21020560

12. Ye Y, Zhu YT, Xin XY, Zhang JC, Zhang HL Li D. Efficacy of Chinese herbal medicine for tPA thrombolysis in experimental stroke: a systematic review and meta-analysis. *Phytomedicine.* (2022) 100:154072. doi: 10.1016/j.phymed.2022.154072

13. Zhu W, Ye Y, Liu Y, Wang XR, Shi GX, Zhang S, et al. Mechanisms of acupuncture therapy for cerebral ischemia: an evidence-based review of clinical and animal studies on cerebral ischemia. *J Neuroimmune Pharmacol.* (2017) 12:575–92. doi: 10.1007/s11481-017-9747-4

14. Ye Y, Li H, Yang JW, Wang XR, Shi GX, Yan CQ, et al. Acupuncture attenuated vascular dementia-induced hippocampal long-term potentiation impairments via activation of D1/D5 receptors. *Stroke.* (2017) 48:1044–51. doi: 10.1161/STROKEAHA.116.014696

15. Liu S, Zhang CS, Cai Y, Guo X, Zhang AL, Xue CC, et al. Acupuncture for post-stroke shoulder-hand syndrome: a systematic review and meta-analysis. *Front Neurol.* (2019) 10:433. doi: 10.3389/fneur.2019.00433

16. Lee CH, Jeon SH, Kim MJ, Ra GD, Lee YH, Hong SH, et al. Factors affecting post-stroke depression in acute ischemic stroke patients after 3 months. *J Pers Med.* (2021) 11:1178. doi: 10.3390/jpm11111178

17. Selvarajah D, Kar D, Khunti K, Davies MJ, Scott AR, Walker J, et al. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. *Lancet Diabetes Endocrinol.* (2019) 7:938–48. doi: 10.1016/S2213-8587(19)30081-6

frontiers | Frontiers in Neurology

TYPE Systematic Review
PUBLISHED 26 September 2022
DOI 10.3389/fneur.2022.1006830

OPEN ACCESS

EDITED BY
Lingyong Xiao,
First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China

REVIEWED BY
Timothy Daly,
Sorbonne Universités, France
Kun Xiong,
Central South University, China
Lv Xie,
Shanghai Jiao Tong University, China
Ruoyu Yang,
Shanghai University of Medicine and Health Sciences, China

*CORRESPONDENCE
Lu Xiao
xiaolu9511@163.com

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 29 July 2022
ACCEPTED 30 August 2022
PUBLISHED 26 September 2022

CITATION
Zhou R, Xiao L, Xiao W, Yi Y, Wen H and Wang H (2022) Bibliometric review of 1992–2022 publications on acupuncture for cognitive impairment. *Front. Neurol.* 13:1006830. doi: 10.3389/fneur.2022.1006830

Bibliometric review of 1992–2022 publications on acupuncture for cognitive impairment

Runjin Zhou[1†], Lu Xiao[2*†], Wei Xiao[2], Yanfei Yi[2], Huanhuan Wen[2] and Hongda Wang[2]

[1]Medical College of Acupuncture-Moxibustion and Rehabilitation, Guangzhou University of Chinese Medicine, Guangzhou, China, [2]Ganzhou Cancer Hospital, Ganzhou, China

Objective: To explore the development context, research hotspots, and frontiers of acupuncture therapy for cognitive impairment (CI) from 1992 to 2022 by visualization analysis.

Methods: Articles about acupuncture therapy for cognitive impairment were retrieved from the Web of Science Core Collection (WoSCC) until 1 March 2022. Basic information was collected by Excel 2007, and VOSviewer 1.6.17 was used to analyze the co-occurrence of countries, institutes, and authors. Co-citation maps of authors and references were analyzed by CiteSpace V.5.8.R3. In addition, CiteSpace was used to analyze keyword clusters and forecast research frontiers.

Results: A total of 279 articles were retrieved, including articles from 19 countries, 334 research institutes, and 101 academic journals. The most published country and institutes were the People's Republic of China (217) and the Fujian University of Traditional Chinese Medicine (40). Ronald C Petersen owned the highest co-citations (56). Keywords and co-cited references cluster showed the main research directions in this area, including "ischemic stroke," "cerebral ischemia/reperfusion," "mild cognitive impairment," "Alzheimer's disease," "vascular dementia," "vascular cognitive impairment with no dementia," "multi-infarct dementia," "synaptic injury," "functional MRI," "glucose metabolism," "NMDA," "nuclear factor-kappa b pathway," "neurotrophic factor," "matrix metalloproteinase-2 (MMP-2)," "tumor necrosis factor-alpha," "Bax," "Caspase-3," and "Noxa". Trending keywords may indicate frontier topics, such as "randomized controlled trial," "rat model," and "meta-analysis."

Conclusion: This research provides valuable information for the study of acupuncture. Diseases focus on mild cognitive impairment (MCI), Alzheimer's disease (AD), and vascular dementia (VaD). Tauopathies with hyperphosphorylation of Tau protein as the main lesions also need to be paid attention to. The development of functional magnetic resonance imaging (fMRI) will better explain the therapeutic effect of acupuncture treatment. The effect of acupuncture on a single point is more convincing, and acupuncture

on Baihui (GV20) may be needed in the future. Finally, the implementation of high-quality multicenter randomized controlled trials (RCTs) requires increased collaboration among experts from multiple fields and countries.

KEYWORDS

acupuncture therapy, cognitive impairment, VOSviewer, CiteSpace, scientometric analysis

Introduction

Cognitive function refers to all kinds of conscious mental activities that human beings always have in the state of awakening, such as simple determination, perception, understanding, and judgment of themselves and the environment to complete complex mathematical calculations (1, 2). Cognitive impairment (CI) is a pathological process in which learning, memory, and thinking judgment related to the brain's advanced intelligent processing are abnormal, resulting in learning and memory disorders, accompanied by aphasia, apraxia, agnosia, and other changes, from mild cognitive impairment (MCI) to dementia (1, 2). MCI is a symptomatic diagnosis in which a patient has a memory or cognitive dysfunction that does not significantly affect daily functioning and does not reach the level of dementia (3). It is an intermediate state between normal aging and dementia, and the prevalence of MCI in adults 65 years and older ranges from 3 to 20%, with more than half progressing to dementia within 5 years (4, 5). Dementia is an acquired intelligence impairment syndrome with cognitive impairment as its core symptom. Cognitive impairment involves memory, learning, orientation, understanding, judgment, computation, language, visual space, and other functions (6). At present, the international diagnosis of dementia includes the American Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (1) and the International Classification of Diseases, 11th Revision (ICD-11) (2). According to the lesion location, it can be divided into cortical dementia (Alzheimer's disease and frontotemporal degeneration), subcortical dementia (vascular dementia [VaD]), mixed cortical and subcortical dementia (multiple infarct dementia, infectious dementia, poisoning, and metabolic encephalopathy), and other dementias (post-traumatic brain injury dementia, etc.).

Of these, Alzheimer's disease (AD) has the highest incidence, and the number of patients in the world has reached 50 million, which is expected to increase to 152 million in 2050, according to the World Alzheimer Report 2019. Patients with AD have decreased the ability of daily living and abnormal mental behavior, posing a significant burden on family caregivers and society (7). It is necessary to find an effective, safe, and inexpensive treatment for older patients. In 2019, the 72nd World Health Assembly passed the ICD-11, which includes traditional Chinese medicine for the first time. Acupuncture is widely used in Asia as one of the non-pharmacological interventions of traditional Chinese medicine. The results of case-control trials based on functional magnetic resonance imaging (fMRI) showed that acupuncture at the Taixi (KI3) acupoint could activate neurons in the cerebral cortex related to cognition, providing imaging evidence support for clinical treatment of CI (8, 9). Randomized controlled trials (RCTs) suggested that acupuncture could improve cognitive function in patients with mild to moderate cognitive impairment (10, 11). In addition, animal experiments are trying to clarify the mechanism of acupuncture (12, 13).

Acupuncture is a technique that has been practiced for thousands of years, and its description can be traced back to a book called *The Huangdi's Internal Classic,* dating from the Han Dynasty (14). During the operation, Deqi is generated by needles into specific acupoints of the human body, so as to achieve the effect of treating diseases (15).

In recent years, more and more research articles on acupuncture have been published. Currently, the reporting quality of RCTs of acupuncture for MCI is moderate to low (16, 17). Most clinical trials did not mention allocation concealment and blinding, nor did they strictly followed the Consolidated Standards for Reporting of Trials (CONSORT) statement and Standards for Reporting Interventions in Controlled Trials of Acupuncture (STRICTA). There is currently a lack of systematic review of trends in this field. Bibliometric visualization analysis is a quantitative analysis method that combines mathematics and statistical methods. It can intuitively highlight the quantitative characteristics of research articles in a certain field and help researchers grasp the development characteristics of the field over time (18). Therefore, this study adopts bibliometric analysis to conduct a systematic review of the application of acupuncture therapy to CI research, aiming to understand the cooperative network, and evaluate the research trends and frontiers.

Materials and methods

Data source and search strategy

Article retrieval was conducted on the Science Citation Index-Expanded (SCI-E) of the Web of Science Core Collection (WoSCC) on 20 March 2022. WoSCC is a relatively comprehensive citation database, and considering that it has the highest applicability with CiteSpace software, so WoSCC was chosen as the preferred retrieval database in this study. The terms "Acupuncture" and "Cognitive Impairment" were used in the MeSH (https://www.ncbi.nlm.nih.gov/mesh) search. The data retrieval strategy is as follows: TS = (Acupuncture OR Pharmacopuncture OR Acupressure OR Acupuncture Therapy OR Acupuncture Point* OR acupunct* OR needl* OR Electroacupuncture OR Ear Acupuncture OR Auricular* OR meridian* OR acupoint*) AND TS= (Cognitive Dysfunction OR Cognitive Impairment OR Neurocognitive Disorder OR Cognitive Decline OR Mild cognitive impairment OR Alzheimer's disease OR dementia OR Vascular dementia). The time span: 01-01-1992 to 01-03-2022.

Inclusion/exclusion criteria

Inclusion criteria

The article type is mainly articles in English, and the research type includes randomized controlled trials (RCTs), retrospective studies, case reports, and animal experiments. The main study population is patients with CI, and the intervention is acupuncture therapy.

Exclusion criteria

Secondary article, such as reviews and meta-analyses, were excluded. In addition, book chapters, letters, editorial material, and meeting abstracts were excluded. At the same time, by reading the abstracts and full texts, researchers should also exclude articles that only briefly mention acupuncture therapy without involving specific therapeutic effects and mechanisms.

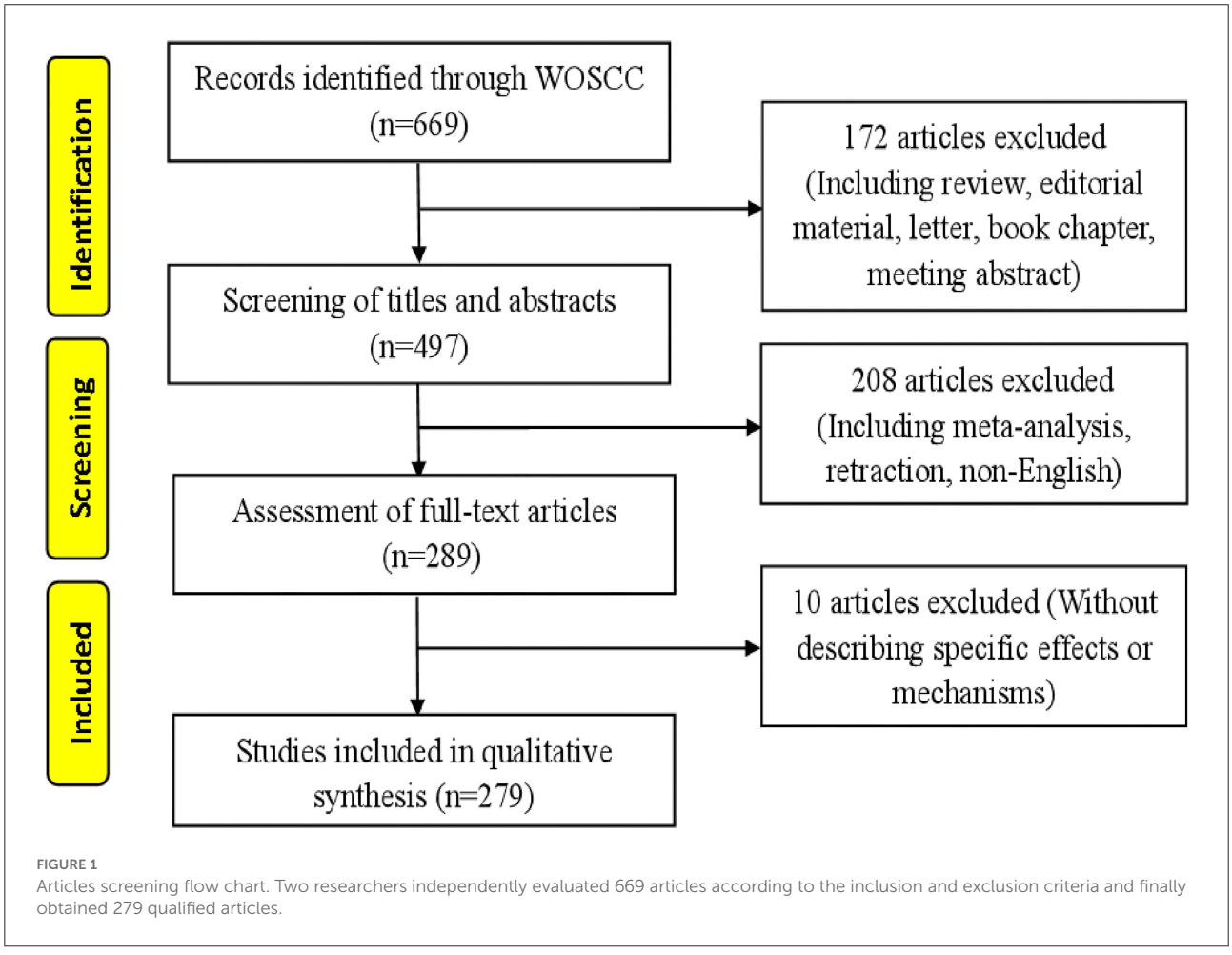


FIGURE 1
Articles screening flow chart. Two researchers independently evaluated 669 articles according to the inclusion and exclusion criteria and finally obtained 279 qualified articles.

Data collection

Raw data from WoSCC were downloaded and verified by two members (LX and RJZ), respectively. The flow chart of research inclusion is shown in Figure 1. The data were then imported into CiteSpace V.5.8.R3 (Drexel University, Philadelphia, PA, USA) and VOSviewer 1.6.17 (Leiden University, Van Eck NJ). The information generated by the software is imported into Excel 2007 (Redmond, WA, USA).

Statistical methods

The WoSCC database was used to analyze the characteristics of the article, such as the number of annual publications and citations, journal sources, research areas, H-index, and impact factor. VOSviewer is a bibliometric analysis software jointly developed by Nees Jan van Eck and Ludo Waltman for mapping scientific knowledge (19). It was used to analyze the co-occurrence of countries/regions, institutes, and authors (which were based on the first author). There were three types of mapping generated: Network Visualization, Overlay Visualization, and Density Visualization. For the Density Visualization, darker yellow indicated more important research. CiteSpace is a tool for visualizing and analyzing trends and patterns in scientific articles (20, 21). It was used for reference/author co-citation analysis, we define that the nodes in the co-citation knowledge graph represent different documents, and the size of the node is proportional to the number of references cited in a specific period. Similarly, a connection between nodes indicates the degree of relationship, the thicker the line, the stronger the connection. Co-occurrence of keywords was analyzed by CiteSpace. We analyzed the characteristics related to keyword clusters, in which the purple reference ring represented the research's high mediating centrality, which played a role in connecting various documents, and the orange represented the newly emerging research (22). This will make it more intuitive to observe the trend of various research hotspots over time.

Results

Annual publications and citations

In total, 279 articles were included from 1992 ($n = 1$) to 2021 ($n = 37$), the citations of these articles also increased rapidly from 2003 ($n = 1$) to 2021 ($n = 781$), with a total of 3,869 citations (Figure 2).

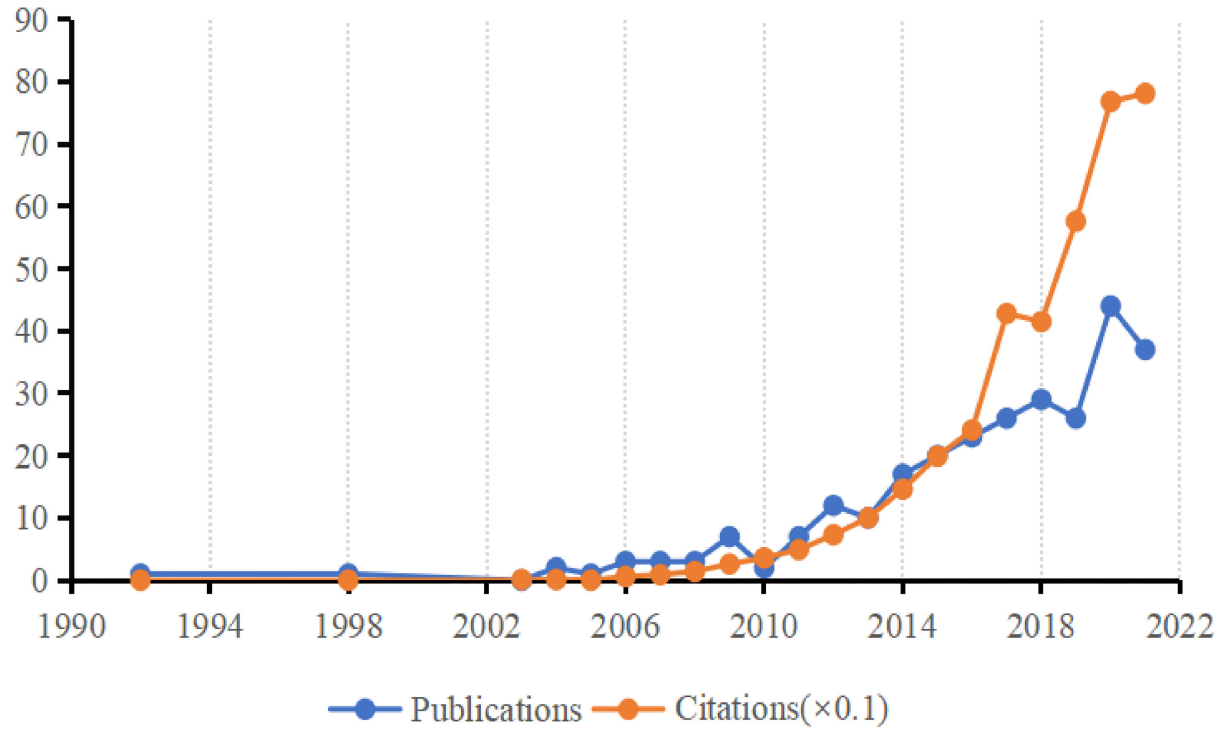


FIGURE 2
Sum of publications and citations. The number of publications and citations from 1992 to 2021 is described by a line chart, where blue represent the number of publications and yellow represent the number of citations (×0.1).

Research areas analysis

A total of 33 research areas were represented. Neurosciences and Neurology ($n = 101$), Integrative Complementary Medicine ($n = 71$), and Research Experimental Medicine ($n = 44$) occupied the main position. Figure 3 shows the top 10 research areas in acupuncture therapy for cognitive impairment.

Distribution of journals and highly cited articles

A total of 101 academic journals have published publications on acupuncture therapy for cognitive impairment. Table 1 lists the top 10 journals with a total of 137 articles. *Evidence-based Complementary and Alternative Medicine* published the most articles ($n = 23$), followed by *Trials* ($n = 18$) and *Neural Regeneration Research* ($n = 14$). Figure 4 displayed the dual-map overlay of journals (23), the left and right sides corresponded to the citation map and the cited journal map, respectively. These labels represented the disciplines covered by the journal. Lines on the map start from the left and end on the right, representing citation links. There were three citation paths: molecular/biology/immunology journals represented by the yellow path, medicine/medical/clinical journals represented by the green path, and neurology/sports/ophthalmology journals represented by the pink path are cited in molecular/biology/genetics areas. Table 2 shows the 10 most frequently cited articles.

Distribution of countries and institutes

A total of 19 countries/regions have published research publications on acupuncture therapy for cognitive impairment, and extensive cooperation between countries/regions has been observed (Figure 5). Table 3 lists the top 10 countries/regions in the number of publications, of which China is the most,

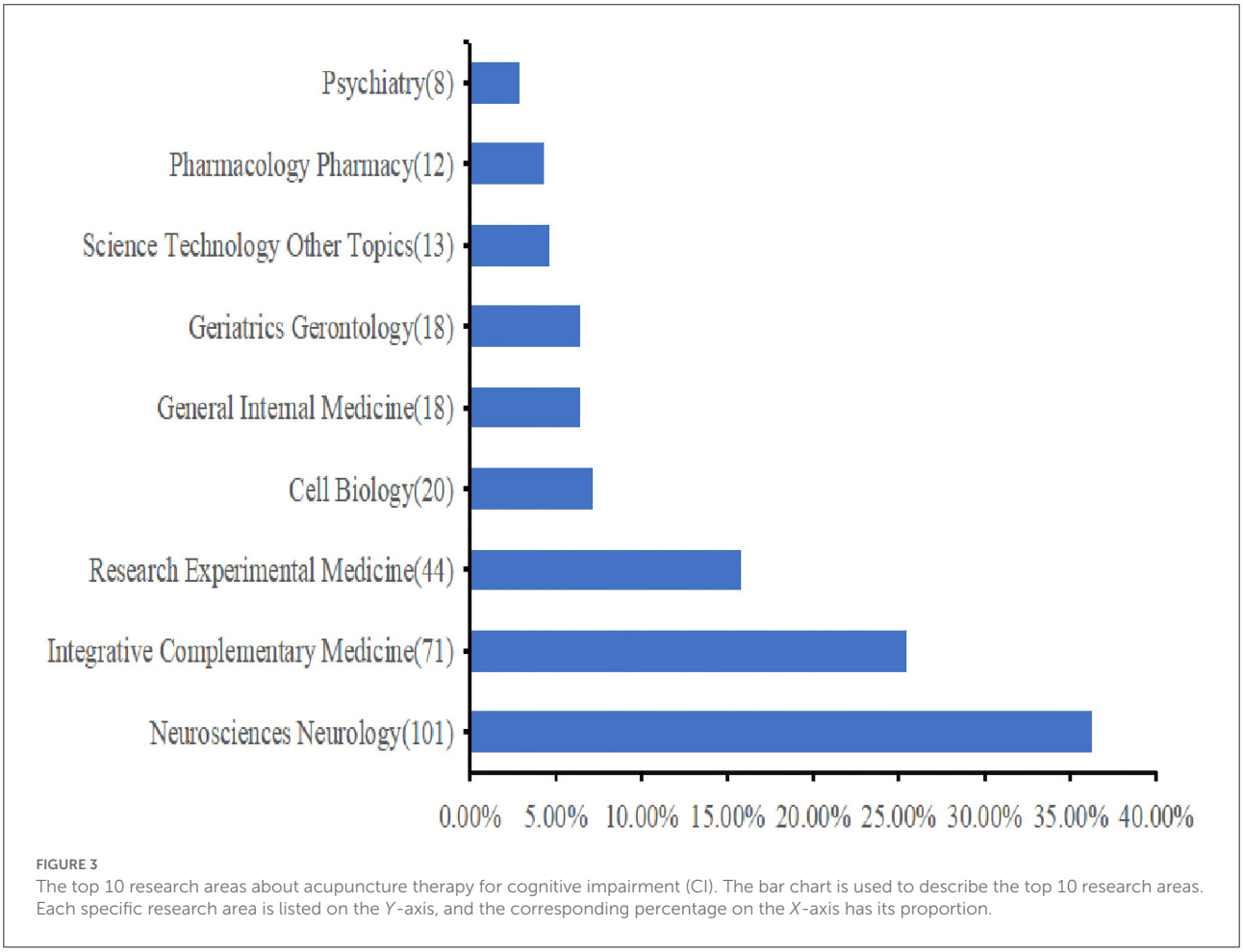


FIGURE 3
The top 10 research areas about acupuncture therapy for cognitive impairment (CI). The bar chart is used to describe the top 10 research areas. Each specific research area is listed on the *Y*-axis, and the corresponding percentage on the *X*-axis has its proportion.

followed by South Korea, the United States, and England. At present, the research on acupuncture treatment of CI is mainly concentrated in China and South Korea. With the increase of international exchanges in recent years, the United States and England are gradually increasing their participation in research, and the multi-country cooperation model is gradually being carried out.

In total, 334 institutes participated in acupuncture research (Figure 5). Table 3 lists the top 10 institutes in terms of publications. It should be noted that we have merged different names of the same institutes, such as Fujian University of TCM merged into Fujian University of Traditional Chinese Medicine, Guangzhou University of Traditional Chinese Medicine merged into Guangzhou University of Chinese Medicine. Statistics showed that the top 10 institutes account for 73.48% of total publications, among which Fujian University of Traditional Chinese Medicine has the largest publications, followed by Capital Medical University and Beijing University of Chinese Medicine.

Analysis of citations and H-index

China ranked first among the top five productive countries in terms of the total number of citations and H-index, followed by South Korea, the United States, England, and Italy (Figure 6). All countries have not contributed to the ESI top articles. High-impact research achievements are still needed in this field.

TABLE 1 The top 10 journals that published articles.

Rank	Journal	Country	Count	IF 2022
1	Evidence-based Complementary and Alternative Medicine	England	23	2.650
2	Trials	England	18	2.728
3	Neural Regeneration Research	China	14	6.058
4	Acupuncture in Medicine	England	12	1.976
5	Medicine	United States	10	1.817
6	BMC Complementary and Alternative Medicine	England	9	4.782
7	Frontiers in Aging Neuroscience	Switzerland	9	5.702
8	Journal of Traditional Chinese Medicine	China	8	2.547
9	Neural Plasticity	United States	8	3.144
10	Neuroscience Letters	Netherlands	8	3.197

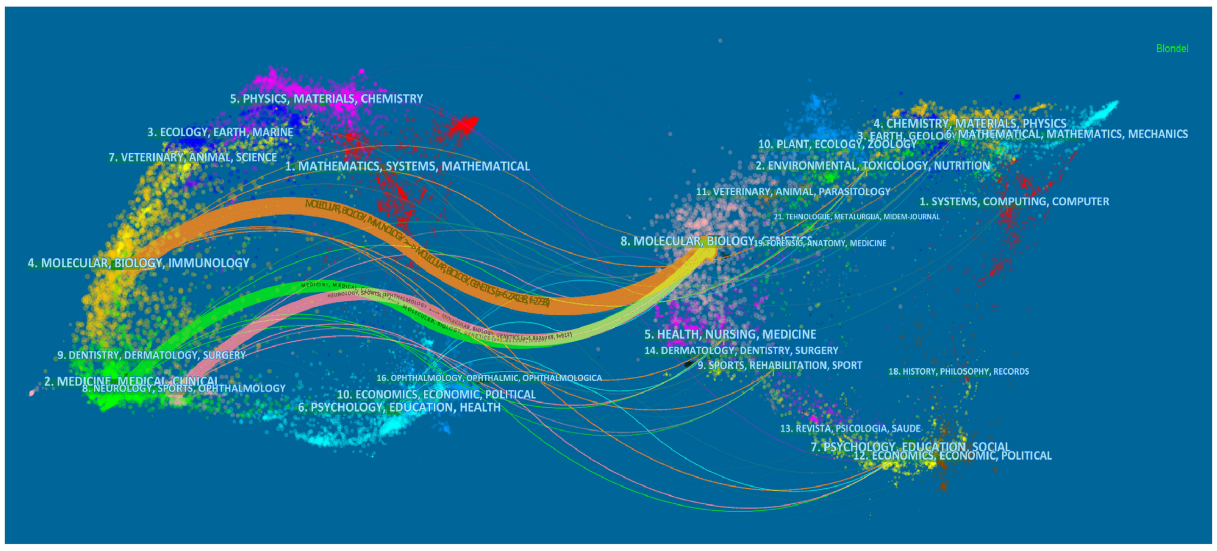


FIGURE 4
The dual-map overlay of journals. The left side correspond to the citation map and the right side represent the cited journal map. Molecular/biology/immunology journals represented by the yellow path, medicine/ medical/ clinical journals represented by the green path, and neurology/sports/ophthalmology journals represented by the pink path are cited in molecular/biology/genetics areas.

TABLE 2 Top 10 most cited articles.

Rank	First/corresponding author	Title	Journal	Cited	Highlight
1	Zhi-qun Wang/ Kun-cheng Li	Acupuncture Modulates Resting State Hippocampal Functional Connectivity in Alzheimer Disease	PLoS One	121	The study clarified that acupuncture at LR3 and LI4 could enhance the hippocampal connectivity in AD patients using fMRI
2	Yuan-yuan Feng/ Jie Tian	FMRI connectivity analysis of acupuncture effects on the whole brain network in mild cognitive impairment patients	Magnetic Resonance Imaging	75	The fMRI study showed that the correlations related to the temporal regions were enhanced in the poststimulus resting brain in MCI patients compared with healthy controls. Compared to superficial acupuncture at KI3, significantly increased correlations related to the temporal regions were found for the deep acupuncture condition.
3	Hai-yan Cheng/ Jing-Xian Han	Acupuncture improves cognitive deficits and regulates the brain cell proliferation of SAMP8 mice	Neuroscience Letters	73	The cognitive deficit of SAMP8 was revealed and significantly improved by "Yiqitiaoxue and Fubenpeiyuan" acupuncture (Sanjiao acupuncture). The experiment observed that the decreased cell proliferation in the dentate gyrus of SAMP8 was greatly enhanced by therapeutic acupuncture. a stream-like distribution of newly proliferated cells presented along the dorsum of alveus hippocampi, extending from the left ventricular to the corpus callosum.
4	Xiao-dong Feng/ Li-dian Chen	Electroacupuncture ameliorates cognitive impairment through inhibition of NF-kappa B-mediated neuronal cell apoptosis in cerebral ischemia-reperfusion injured rats	Molecular Medicine Reports	68	Electroacupuncture at GV20 and GV24 suppressed the I/R-induced activation of NF-κB signaling in ischemic cerebral tissues, which led to the inhibition of cerebral cell apoptosis. Furthermore, electroacupuncture markedly downregulated the expression of pro-apoptotic Bax and Fas, two critical downstream target genes of the NF-κB pathway.
5	Zhi-qun Wang/ Kun-cheng Li	Effect of Acupuncture in Mild Cognitive Impairment and Alzheimer Disease: A Functional MRI Study	PLoS One	66	To clarify the mechanisms of acupuncture at Tai LR3 and LI4 in treating MCI and AD patients by using fMRI.
6	Xu-ying Li/ Li-ze Xiong	Electroacupuncture decreases cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice	BMC Complementary and Alternative Medicine	64	Electroacupuncture stimulation at GV20 significantly ameliorated the learning and memory deficits of APP/PS1 mice, decreased Aβ deposits, and increased brain-derived neurotrophic factor (BDNF) expression and neurogenesis in the hippocampus and cortex of EA-treated AD mice were detected.
7	Cun-Zhi Liu/ Jing-Xian Han	Acupuncture prevents cognitive deficits and oxidative stress in cerebral multi-infarction rats	Neuroscience Letters	63	suggesting that acupunctural prescription including CV17, CV12, CV6, ST36, and SP10 ameliorated oxidative injuries induced by cerebral multi-infarction by increasing the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the hippocampus.
8	Jian-chun Yu/ Jing-Xian Han	Acupuncture improved cognitive impairment caused by multi-infarct dementia in rats	Physiology & Behavior	62	The pattern of multi-infarct dementia in rats was made by injecting homogeneous emboli into the internal carotid artery. Acupunctural prescription including CV17, CV12, CV6, ST36, and SP10, the present results suggested that acupuncture exerted a protective effect on cognitive impairment caused by cerebral multi-infarction in rats, and acupuncture has a specificity of cure.

(Continued)

TABLE 2 (Continued)

Rank	First/corresponding author	Title	Journal	Cited	Highlight
9	Bombi Lee/ Hyejung Lee	Acupuncture stimulation improves scopolamine-induced cognitive impairment *via* activation of cholinergic system and regulation of BDNF and CREB expressions in rats	BMC Complementary and Alternative Medicine	57	This study aimed to examine whether acupuncture stimulation at GV20 improves memory defects caused by scopolamine (SCO) administration in rats. The result showed that acupuncture significantly alleviated memory-associated decreases in the levels of choline acetyltransferase (ChAT), BDNF, and cAMP-response element-binding protein (CREB) proteins in the hippocampus. Moreover, acupuncture restored the expression of choline transporter 1 (CHT1), vesicular acetylcholine transporter (VAChT), BDNF, and CREB mRNA in the hippocampus.
10	Li-Chan Lin	Using Acupressure and Montessori-Based Activities to Decrease Agitation for Residents with Dementia: A Cross-Over Trial	Journal of the American Geriatrics Society	55	A double-blinded, randomized cross-over design was used to evaluate the effectiveness of acupressure and Montessori-based activities in decreasing the agitated behaviors of residents with dementia. Results mainly demonstrated that the acupressure and Montessori-based activities groups saw a significant decrease in agitated behaviors, aggressive behaviors, and physically nonaggressive behaviors than the presence group.

Analysis of authors

Over 1,210 authors contributed to acupuncture research. The co-occurrence map of authors is shown in Figure 5. Table 4 lists the top 10 authors in the number of publications. Li-Dian Chen and Cun-Zhi Liu (26 publications) were both ranked first, followed by Jing Tao (25 publications) and Jia Huang (19 publications).

The co-citation of authors was analyzed by CiteSpace (Figure 7A). Among the top 10 co-cited authors (Table 4), Ronald C Petersen (56 co-citations) ranked first, followed by Zhi-Qun Wang (53 co-citations), and Jian-Chun Yu (46 co-citations). The top 17 authors with the strongest citation bursts are listed in Figure 8A, and the beginning to the end of each burst interval is indicated by a red line. Jing Zhou (strength 4.38, 2017–2022), Meng Zhang (strength 4.43, 2019–2022), Min Deng (strength 4.40, 2019–2022), Nasreddine ZS (strength 4.38, 2017–2022), Yang-Juan Jia (strength 4.08, 2019–2022), and Jing Jiang (strength 4.08, 2019–2022) have been cited in recent 3 years, indicating that the authors have been active in this field in recent years. New research has been published in the study of cognitive impairment.

Analysis of co-cited references

We used CiteSpace to analyze the co-citation of references (Figure 7B). Table 5 lists the top 10 co-cited references. The co-cited reference clusters are shown in Figure 9A. The network contained 592 nodes and 1,535 links. The Modularity Q was 0.8741 (>0.5), meaning that the clusters of networks were reasonable, and the Mean Silhouette S was 0.9285 (>0.5), indicating that the homogeneity of clusters were acceptable (20). In this network, more important clustering labels were listed in 20 clusters: #0 vascular dementia, #1 ischemic stroke, #2 NMDA, #3 nerve regeneration, #4 mild cognitive impairment, #5 Alzheimer's disease, #6 nuclear factor-kappa b pathway, #7 tumor necrosis factor-alpha, #8 synaptic injury, #9 Bax, #11 energy metabolism, #12 multi-infarct dementia, #13 vascular cognitive impairment with no dementia, #16 waiguan (SJ5), and #19 Caspase-3. The top 13 co-cited references with the strongest citation bursts are listed in Figure 8B.

Analysis of keywords

CiteSpace was used to analyze the co-occurrence of keywords (Figure 10). Table 4 lists the top 10 keywords. Co-cited keyword clusters network contained 375 nodes and 1,006 links. the Modularity Q was 0.7254, and the Mean Silhouette S was 0.9044 (>0.5) (Figure 9B). There were 16 clustering labels listed: #0 cerebral ischemia/reperfusion, #1 memory, #2 brain, #3 clinical trial, #4 randomized controlled trial, #5 vascular dementia, #6 traditional Chinese medicine, #7 rehacom training, #8 functional MRI, #9 cognitive deficits,

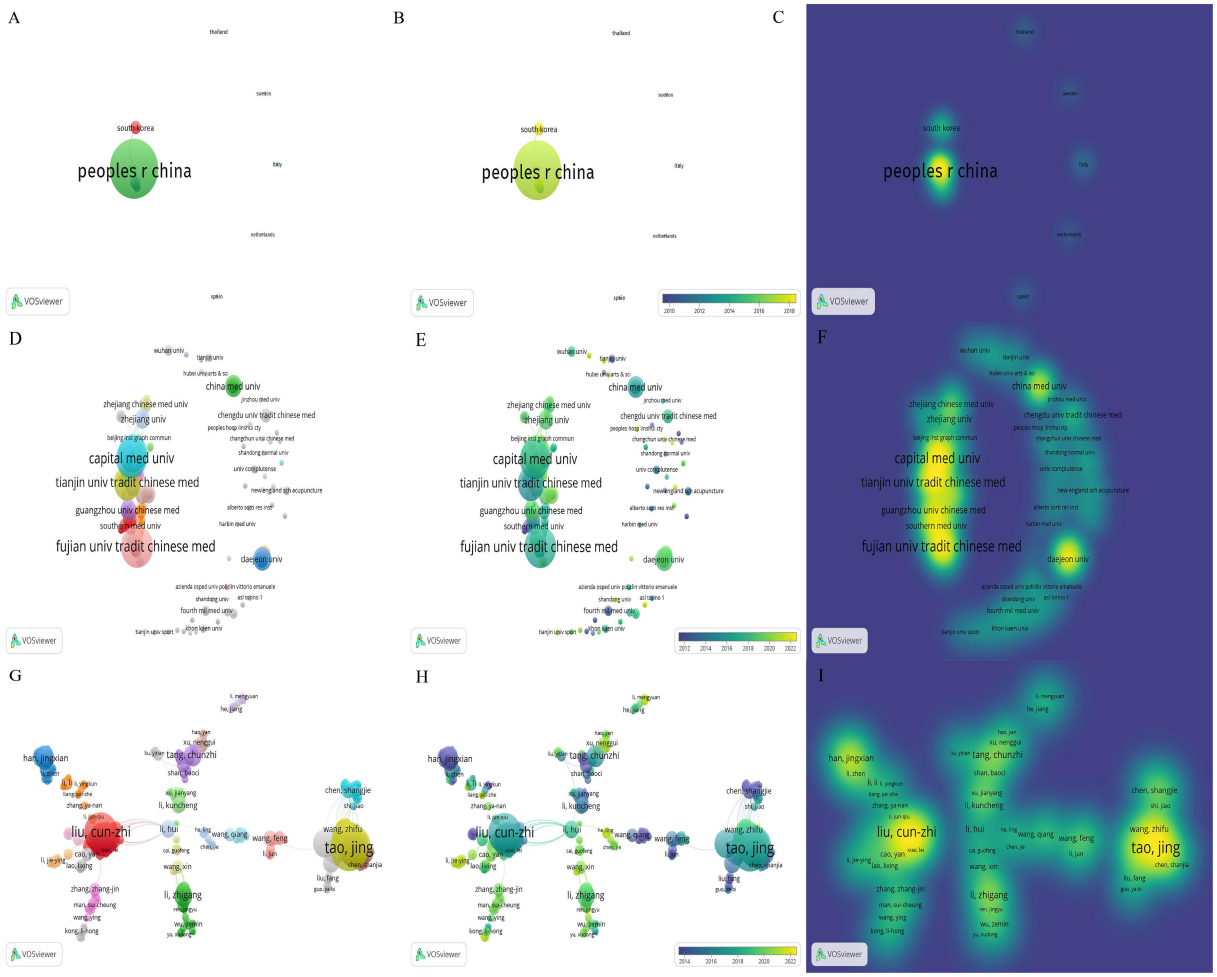


FIGURE 5
The network map of countries/regions, institutes, and active authors. **(A)** The Network Visualization map of countries/regions, **(B)** the Overlay Visualization map of countries/regions, and **(C)** the Density Visualization map of countries/regions. **(D)** The Network Visualization map of institutes, **(E)** the Overlay Visualization map of institutes, and **(F)** the Density Visualization map of institutes. **(G)** The Network Visualization map of active authors, **(H)** the Overlay Visualization map of active authors, and **(I)** the Density Visualization map of active authors.

TABLE 3 Top 10 countries/region and institutions in the number of publications.

Rank	Country/region	Count	Institute	Count
1	China	217	Fujian University of Traditional Chinese Medicine	40
2	South Korea	26	Capital Medical University	34
3	the United States	24	Beijing University of Chinese Medicine	31
4	England	5	Tianjin University of Traditional Chinese Medicine	26
5	Italy	4	Guangzhou University of Chinese Medicine	17
6	Australia	2	Korea Institute of Oriental Medicine	14
7	Canada	2	China Medical University (Taiwan)	13
8	Germany	2	Shanghai University of Traditional Chinese Medicine	11
9	Norway	2	Southern Medical University	10
10	Spain	2	Chinese Academy of Sciences	9

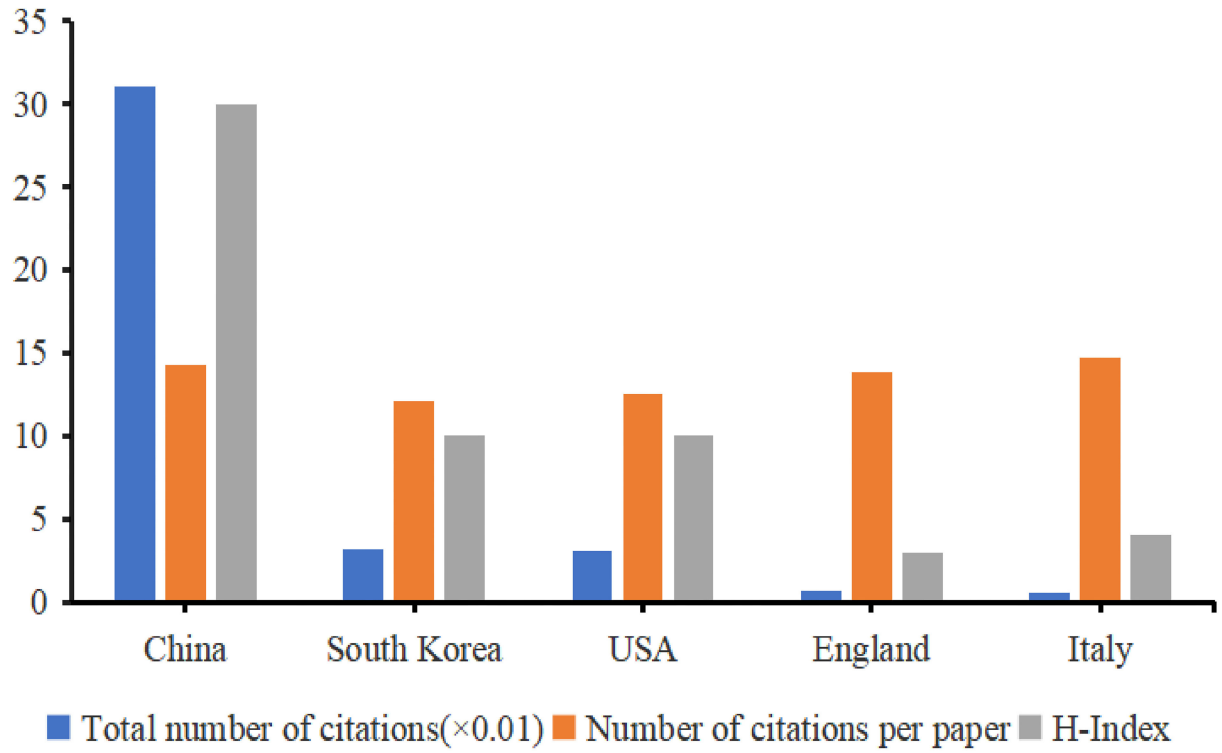


FIGURE 6
The distribution of citation (×0.01), number of citations per article, and H-index in the top 5 countries. According to the number of citations and H-index, the histogram intuitively shows the top 5 countries, among which Chinese scholars have done the most research in this field and have the highest influence.

TABLE 4 The top 10 authors, co-cited authors, and keywords.

Rank	Author	Count	Co-cited Author	Count	keyword	Count
1	Li-Dian Chen	26	Ronald C Petersen	56	Alzheimer's disease	102
2	Cun-Zhi Liu	26	Zhi-Qun Wang	53	mild cognitive impairment	97
3	Jing Tao	25	Jian-Chun Yu	46	acupuncture	57
4	Jia Huang	19	Li-Jun Bai	42	vascular dementia	45
5	Wei-Lin Liu	15	Lan Zhao	38	dementia	43
6	Jing-Wen Yang	15	Yuan-yuan Feng	34	memory	34
7	Jing-Xian Han	12	Ru-Hui Lin	33	brain	33
8	Ru-Hui Lin	12	Jing Zhou	33	stroke	30
9	Xue-Rui Wang	12	Cun-Zhi Liu	29	activation	30
10	Jian-Chun Yu	12	Guang-Xia Shi	29	expression	27

#10 protein, #11 glucose metabolism, #12 neurotrophic factor, #13 dentate gyrus, #14 matrix metalloproteinase-2 (MMP-2), and #15 Noxa. The top 23 co-cited keywords with the strongest citation bursts are listed in Figure 8C. As shown in the figure, the part selected by the red square represents the highly cited keywords in recent years, which are mainly reflected in randomized controlled trials, meta-analysis, and the construction of animal models.

Discussion

General information

The number of publications and citations has shown a rapid upward trend every year, indicating that acupuncture therapy for MCI research is still attracting attention. Among the top 10 contributing countries, China and South Korea accounted

FIGURE 7
The co-citation map of authors and references. **(A)** The co-citation map of authors and **(B)** the co-citation map of authors references.

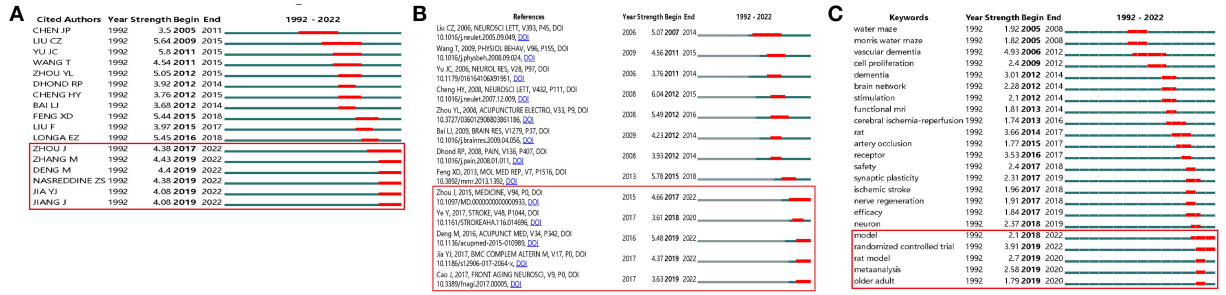


FIGURE 8
The co-cited authors, co-cited references, and keyword with the strongest citation bursts. **(A)** The co-cited authors with the strongest citation bursts, **(B)** the co-cited references with the strongest citation bursts, and **(C)** the keyword with the strongest citation bursts. Among them, the co-cited authors, co-cited references, and keywords in the red box are the most obvious in the past 3 years, which may be the latest research hotspots.

TABLE 5 The top 10 co-cited references.

Rank	Co-cited reference	Title	Count
1	Zhou J, 2015, MEDICINE, V94, P0, DOI 10.1097/MD.0000000000000933	The effectiveness and safety of acupuncture for patients with Alzheimer disease: a systematic review and meta-analysis of randomized controlled trials	24
2	Wang ZQ, 2012, PLOS ONE, V7, P0, DOI 10.1371/journal.pone.0042730	Effect of acupuncture in mild cognitive impairment and Alzheimer disease: a functional MRI study	23
3	Feng YY, 2012, MAGN RESON IMAGING, V30, P672, DOI 10.1016/j.mri.2012.01.003	FMRI connectivity analysis of acupuncture effects on the whole brain network in mild cognitive impairment patients	22
4	Feng XD, 2013, MOL MED REP, V7, P1516, DOI 10.3892/mmr.2013.1392	Electroacupuncture ameliorates cognitive impairment through inhibition of NF-κB-mediated neuronal cell apoptosis in cerebral ischemia-reperfusion injured rats	20
5	Li XY, 2014, BMC COMPLEM ALTERN M, V14, P0, DOI 10.1186/1472-6882-14-37	Electroacupuncture decreases cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice	19
6	Deng M, 2016, ACUPUNCT MED, V34, P342, DOI 10.1136/acupmed-2015-010989	Acupuncture for amnestic mild cognitive impairment: a meta-analysis of randomized controlled trials	15
7	Liu F, 2014, J ALTERN COMPLEM MED, V20, P535, DOI 10.1089/acm.2013.0364	A meta-analysis of acupuncture use in the treatment of cognitive impairment after stroke	15
8	Zhou YL, 2008, ACUPUNCTURE ELECTRO, V33, P9, DOI 10.3727/036012908803861186	Effect of acupuncture given at the HT7, ST36, ST40 and KI3 acupoints on various parts of the brains of Alzheimer's disease patients	14
9	Cheng HY, 2008, NEUROSCI LETT, V432, P111, DOI 10.1016/j.neulet.2007.12.009	Acupuncture improves cognitive deficits and regulates the brain cell proliferation of SAMP8 mice	14
10	Li QQ, 2015, PHYSIOL BEHAV, V139, P482, DOI 10.1016/j.physbeh.2014.12.001	Hippocampal cAMP/PKA/CREB is required for neuroprotective effect of acupuncture	13

for the largest proportion (87.10%). Research institutions are also represented by traditional Chinese medicine colleges, which reflects the high acceptance of acupuncture in Asia. In terms of the distribution of authors, Li-Dian Chen ranked first in 26 publications. According to Price's Law (24), the minimum number of publications for core authors is $N = 0.749\sqrt{M\,\mathrm{max}}$ (Mmax is the publications of the most prolific authors), calculated at $N \approx 4$. In terms of the number of articles published, 89 authors have published more than 4 articles, accounting for 7.36% (<50%), indicating that the core author team in this research field has not yet been formed. At present, scholars who study acupuncture for CI are mainly concentrated in Asia and relatively few in Europe and the United States, which may have a certain relationship with the culture they come into contact with. It is necessary to popularize acupuncture and let more people know about it. At the same time, strengthening the cooperation and exchanges between scholars from various countries will help to further explore the effective mechanism of acupuncture

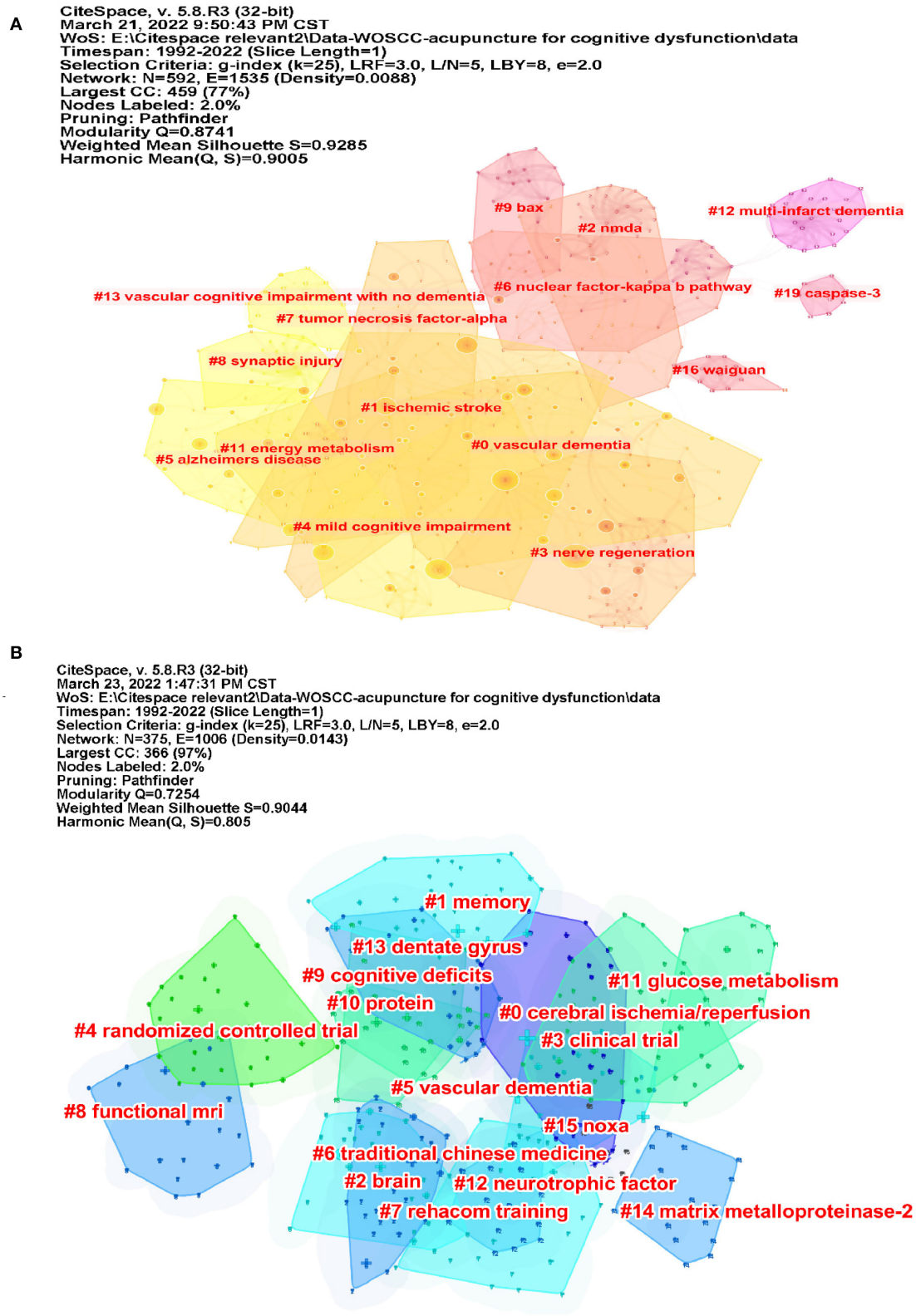


FIGURE 9
The co-cited reference and keyword clusters map for publications. **(A)** The co-cited reference clusters map for publications and **(B)** the keyword clusters map for publications. In clustering, the same elements would be aggregated together, each cluster existed independently, and the cluster label is a name for each cluster diagram.

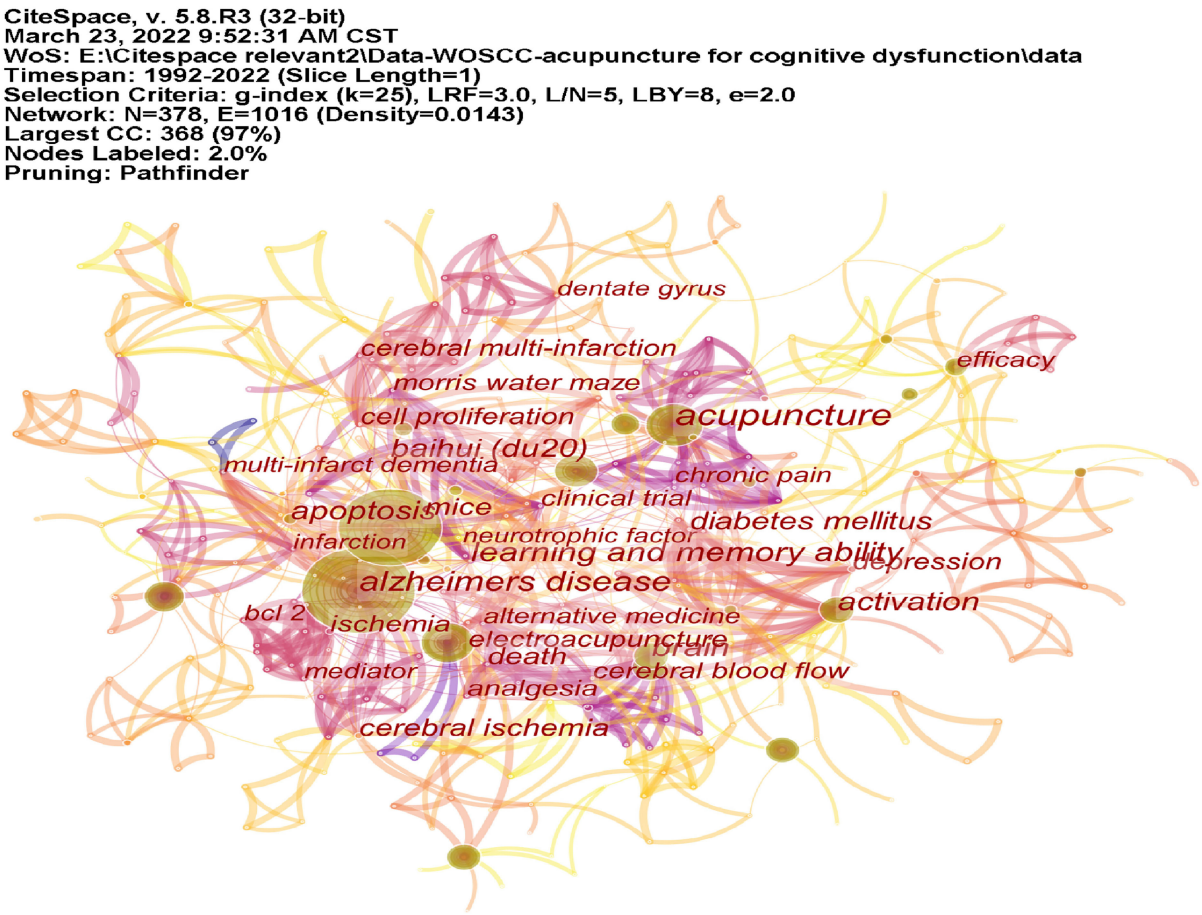


FIGURE 10
The co-occurrence map of keywords. CiteSpace was used to analyze the co-occurrence of keywords. The figure shows that the keywords, such as Alzheimer's disease (AD), acupuncture, electroacupuncture, brain, activation, and Baihui occupied the main position.

treatment. Perhaps this will not only be limited to the study of improving cognitive function.

Research hotspots and frontiers

RCTs of acupuncture therapy for MCI

Earlier identification and intervention of mild cognitive impairment are necessary to delay the progression of the disease to dementia. The results of the meta-analysis based on RCTs suggested that acupuncture is effective in the treatment of patients with MCI and can improve their activity on a daily living scale, the mini-mental state examination (MMSE), and Montreal cognitive assessment scores (MoCA) (25). As mentioned in the preface, the lack of high-quality RCT articles is the main reason leading to the medium-low quality evidence of acupuncture treatment CI. There is still a need for standardized reports on acupuncture research.

Animal models focus on AD and VaD

Alzheimer's disease models involved in this study include the rapid aging models, transgenic models, and Aβ injection models. Senescence-Accelerated Mice include two strains, SAM-P and SAM-R (26, 27). SAM-P exhibits rapid aging, AD-specific Aβ aggregation, and hyperphosphorylation of Tau protein in pathology. SAM-P has 9 sub-strains, among which SAMP8 is an ideal animal model for aging-related learning and memory deficits that are similar to the clinical manifestations of patients with AD (28). SAMR1 mice retain normal aging characteristics and are often used as controls. The transgenic animal models involved in this study are amyloid precursor protein/Presenilin 1 (APP/PS1) mice and five familial mutations (5XFAD) mice. APPSwe/PS1 (B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J) double transgenic mice model. APPSwe is the Swedish mutation of the amyloid precursor protein, whereas PS1 is the mutant form of human presenilin 1 (29). 5XFAD mice overexpress human amyloid precursor protein (APP) and presenilin-1 (PS1)

TABLE 6 The putative mechanisms of acupuncture on different Alzheimer's disease animal models.

Model	Acupuncture point	Mechanism
SAMP8 mice	CV17, CV12, CV6, ST36, SP10	Regulates brain cell proliferation (34). Reduced neuron loss in hippocampal regions CA3 and DG (35). upregulated the expression of bFGF, EGF, and BDNF (36). improving synaptophysin mRNA and protein levels (37). promoting Hsp84 and Hsp86 expression (38). accelerates synaptophysin production (39). Down-regulating PI3K/PDK1/nPKC/Rac1 signaling pathway (40).
	GV14, BL23	increased the levels of p-AMPK (41). upregulated the expression of SIRT1 and PGC-1α (42). downregulation of BACE1(43).
	GV20, GV29	increased CBF in the prefrontal lobe and hippocampus (44). by balancing the gut microbiota (45). enhanced paravascular influx in the glymphatic system inhibited the reactivity of astrocytes and improved AQP4 polarity (46).
	GV20, GV29, GV26	improved the level of glucose metabolism (47).
	GV20, BL23	inhibited the AMPK/eEF2K/eEF2 signaling pathway (48).
	GV20, ST36	downregulated NLRP3/caspase-1 pathway (49).
	GV20, BL23, KI3	inhibited activation of astrocytes and microglia and decreased expression of pro-inflammatory cytokines, TNF-α, and IL-17 (50).
APP/PS1 mice	GV20	up-regulated the expression of BDNF (51, 52). Induced phosphorylated AMPK and AKT inhibited the phosphorylation level of the mammalian target of mTOR (53). suppressed GFAP and NDRG2 upregulation (54). increased the expression levels of BDNF and proBDNF, p-TrkB was upregulated, and p75NTR was decreased (55).
	GV20, GV29, GV26	enhancing glucose metabolism (56, 57). downregulated of BACE1, p-PKA protein (58). inhibited JNK signaling pathway (59). induced AKT (Ser473) and GSK3β (Ser9) phosphorylation, inhibited the phosphorylation of Tau (Ser199 and Ser202) proteins (60).
	GV20, BL23	reduced the expressions of BACE1, and increased the expression of IDE protein (61).
	GV20, GV24	activated AMPK to enhance the process of Aerobic glycolysis (AG), and enhanced glucose metabolism (62).
5x FAD	KI3	inhibition of neuroinflammation and increased glucose metabolism (63). upregulation of synaptophysin and postsynaptic density-95 protein (64).
	GV24, GB13	activated TFEB *via* inhibiting the AKT-MAPK1-MTORC1 pathway (65).
	GV20, GV24	activating the medial septal and vertical limb of the diagonal band and dentate gyrus (MS/VDB-DG) cholinergic neural circuit (66).
injecting $A\beta_{1-40}$ Rat Model	GV20, BL23	activation of PPAR-γ and inhibition of p-p38MAPK expression (67). upregulated the expression of Bcl-2 and downregulated the expression of Bax, downregulated the level of Notch1 and Hes1 mRNA in the hippocampus (68).
injecting $A\beta_{1-42}$ Rat Model	GV20, BL23	downregulated the expression of GSK-3β (69).
	GV29, LI20	the activation level of PI3K/AKT signaling and the phosphorylation inactivation of GSK-3β (70).

Dentate Gyrus (DG), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), brain-derived neurotrophic factor (BDNF), heat shock protein (Hsp), Phosphatidylinositol 3 Kinase (PI3K), Phosphoinositol-Dependent Kinase 1 (PDK1), Novel Protein Kinase C (nPKC), Sirtuin 1 (SIRT1), proliferator-activated receptor-γ-co-activator-1α (PGC-1α), Beta-secretase 1 (BACE1), Cerebral blood flow (CBF), aquaporin-4 (AQP4), AMP-activated protein kinase (AMPK), eukaryotic elongation factor-2 kinase (eEF2K), eukaryotic elongation factor-2 (eEF2), Nod-like receptor family pyrin domain containing 3 (NLRP3), tumor necrosis factor-α (TNF-α), interleukin-17 (IL-17), thymoma viral proto-oncogene (AKT), glial fibrillary acidic protein (GFAP), N-myc downstream-regulated gene 2 (NDRG2), c-Jun N-terminal kinase(JNK), insulin degrading enzyme (IDE), mitogen-activated protein kinase 1 (MAPK1), mechanistic target of rapamycin kinase complex 1 (MTORC1), glycogen synthase kinase-3β (GSK-3β).

mutants, namely, the Swedish (K670 N and M671 L), Florida (I716V), and London (V717I) mutations in APP and the PS1 mutations M146 L and L286 V (30). The above two kinds of transgenic animals can specifically produce excessive APP, which affects the accumulation of Aβ, and finally forms senile plaques (SP). Aβ injection models are used to inject Aβ polypeptide fragments of different lengths into specific brain regions, such as Aβ1-40 (31) and Aβ1-42 (32). It is worth mentioning that all the animal studies on the acupuncture treatment of AD seem to be directed at the regulatory mechanism of Aβ. At present, there are few studies on tauopathies, which we believe can be further studied in the future.

For VaD models, the permanent, bilateral common carotid artery occlusion (2-VO) is most commonly used in this study (33). By ligating the common carotid artery, a chronic cerebral hypoperfusion state is created, thereby causing ischemia

TABLE 7 Mechanisms of acupuncture on different vascular dementia animal models.

Model	Acupuncture point	Mechanism
using the permanent, bilateral common carotid artery occlusion (2VO)	GV20, ST36	Up-regulate the protein and mRNA levels of Nrf2 and its target genes HO-1 and NQO1 (71). up-regulated the expressions of Trx-1 and TrxR-1 and inhibited the activation of the ASK1/JNK/ p38 pathway (72). downregulated the expression of TXNIP, NLRP3, caspase-1, and IL-1β (73). downregulated the expression of TLR4, accompanied by a decrease in microRNA-93 and MyD88/ NF-κB signaling pathway activation. activated of D1/ D5 receptors (74). activated of D1/D5 receptors (75). increased the expression of Hippocampal mitochondrial respiratory complex enzymes (complex I, II, IV) activities and cytochrome c oxidase IV expression (76). increased CBF attenuated the loss of myelin basic protein and microglial accumulation associated with IL-1β and IL-6 production (77). decreased ROS production and improved LTP (78).
	GV20, GV14, BL23	up-regulated expression of mTOR and eIF4E (79). inhibited expression of Noxa and caspase-3 expression (80). blocked expression of p53 and Noxa (81).
	GV20, GV14	promoted synaptophysin expression (82).
four-vessel occlusion (4-VO)	GV20, CV17, BL17, CV6, SP6	inhibited the protein and mRNA expressions of TLR4 and MyD88 in the hippocampus of rats, and reduced the expressions of serum IL-6 and TNF-α (83).
Via bilateral middle cerebral artery occlusion	GV20, GV14, BL23	up-regulated expression of p70 ribosomal protein S6 kinase and ribosomal protein S6 (84).
with 3% microemboli saline suspension	ST36	increased the pyramidal neuron number (85).

Nuclear factor E2 related factor 2 (Nrf2), thioredoxin-1 (Trx-1), thioredoxin reductase-1 (TrxR-1), apoptosis signal-regulating kinase 1 (ASK1), c-Jun N-terminal kinase (JNK), Thioredoxin-interacting protein (TXNIP), interleukin-1β (IL-1β), Toll-like receptors 4 (TLR4), myeloid differentiation factor 88 (MyD88), nuclear factor-kappa B (NF-κB), reactive oxygen species (ROS), long-term potentiation (LTP), rapamycin (mTOR), eukaryotic translation initiation factor 4E (eIF4E), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6).

and hypoxia in brain tissue, especially the hippocampus and cortex. However, this modeling method has a high lethality rate, and the modified version of 2-VO can improve the survival rate.

Various putative mechanisms of acupuncture in the treatment of AD and VaD

The putative mechanism of acupuncture in the treatment of AD and VaD mainly focuses on the repair of neuroinflammation, regulating autophagy and apoptosis, and improving energy metabolism. Tables 6, 7 lists the different putative mechanisms of acupuncture for AD (34–70) and VaD (71–85). It is worth noting that Baihui (GV20, also called DU20) occupies the most acupuncture points, prompting its importance in acupoint selection. Furthermore, the selection of acupuncture points is based on the theory of Chinese medicine, and most of the points are not at least one. The final result makes it difficult to determine whether it is the effect of single point stimulation or superposition of combination. In the experimental grouping, in addition to setting up the sham group, the setting of a single acupoint or combination needs further consideration.

Functional magnetic resonance imaging is one of the important tools to research cognitive function

Functional magnetic resonance imaging is a non-invasive neuroimaging method with a high spatial resolution to study brain function. Its method of detecting functional connectivity of various brain regions in resting or task states may objectively identify cognitive impairment. Acupuncture in the cognitive impairment group compared with healthy controls to observe whether cognitive-related brain areas (such as dentate gyrus, frontotemporal lobe, and frontal lobe) are activated (86–88).

Limitations

This study also has some disadvantages: first, our study was conducted on 20 March 2022, and included all articles up to 01 March 2022, but since the WoSCC database is still open to relevant documents in 2022, this section is omitted. Second, since each published article was limited to 3–10 keywords, some core words in these articles were not included in the bibliometric analysis, so the analysis results may also be affected by incomplete keyword extraction. Finally, as the search was limited to journals indexed in the WoSCC database, some

articles not included in the WOSCC database were left out. Other bibliometric studies have also reported these limitations (89–91).

Conclusion

We analyzed the research progress of acupuncture in the treatment of CI through the visualization analysis, and this study shows the current achievements of acupuncture in the treatment of CI and the possible directions of further research in the future, which will be helpful for clinicians and researchers. The results suggest that diseases focus on MCI, AD, and VaD. Pathologically, the detection of Aβ and APP is the main concern. Tauopathies with hyperphosphorylation of Tau protein as the main lesions also need to be paid attention to. In addition, fMRI is one of the means to elucidate the mechanism of treatment. At the same time, the complexity of acupoint selection makes it difficult to explain the specific pathway mechanism of acupuncture treatment of diseases. We think it is necessary to select a single point, and we found that Baihui (GV20) is the most commonly used, so we suggest that researchers can further elaborate on the mechanism of treating CI with GV20 acupuncture. Finally, the implementation of high-quality multicenter randomized controlled trials requires increased collaboration among experts from multiple fields and countries.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

RZ and LX conceived and designed the experiments, authored, and reviewed drafts of the paper. RZ wrote the original draft. YY, RZ, WX, and LX analyzed the data. LX reviewed and edited the final draft. HWa, YY, RZ, LX, and WX performed the experiments and approved the final draft. HWa, YY, and WX prepared figures and/or tables. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1006830/full#supplementary-material

References

1. Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS, et al. Classifying neurocognitive disorders: the DSM-5 approach. *Nat Rev Neurol.* (2014) 10:634–42. doi: 10.1038/nrneurol.2014.181

2. Reed GM, First MB, Kogan CS, Hyman SE, Gureje O, Gaebel W, et al. Innovations and changes in the ICD-11 classification of mental, behavioural and neurodevelopmental disorders. *World Psychiatry.* (2019) 18:3–19. doi: 10.1002/wps.20611

3. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med.* (2004) 256:183–94. doi: 10.1111/j.1365-2796.2004.01388.x

4. Langa KM, Levine DA. The diagnosis and management of mild cognitive impairment: a clinical review. *JAMA.* (2014) 312:2551–61. doi: 10.1001/jama.2014.13806

5. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, et al. Mild cognitive impairment. *Lancet.* (2006) 367:1262–70. doi: 10.1016/S0140-6736(06)68542-5

6. Ritchie K, LovestONE S. The dementias. *Lancet.* (2002) 360:1759–66. doi: 10.1016/S0140-6736(02)11667-9

7. Association As. (2021 Alzheimer's disease facts and figures. *Alzheimers Dement.* (2021) 17:327–406. doi: 10.1002/alz.12328

8. Jia B, Liu Z, Min B, Wang Z, Zhou A, Li Y, et al. The effects of acupuncture at real or sham acupoints on the intrinsic brain activity in mild cognitive impairment patients. *Evid Based Complement Alternat Med.* (2015) 2015:529675. doi: 10.1155/2015/529675

9. Chen S, Xu M, Li H, Liang J, Yin L, Liu X, et al. Acupuncture at the Taixi (KI3) acupoint activates cerebral neurons in elderly patients with mild cognitive impairment. *Neural Regen Res.* (2014) 9:1163–8. doi: 10.4103/1673-5374.135319

10. Jia Y, Zhang X, Yu J, Han J, Yu T, Shi J, et al. Acupuncture for patients with mild to moderate Alzheimer's disease: a randomized controlled trial. *BMC Complement Altern Med.* (2017) 17:556. doi: 10.1186/s12906-017-2064-x

11. Wang S, Yang H, Zhang J, Zhang B, Liu T, Gan L, et al. Efficacy and safety assessment of acupuncture and nimodipine to treat mild cognitive impairment after cerebral infarction: a randomized controlled trial. *BMC Complement Altern Med.* (2016) 16:361. doi: 10.1186/s12906-016-1337-0

12. Feng X, Yang S, Liu J, Huang J, Peng J, Lin J, et al. Electroacupuncture ameliorates cognitive impairment through inhibition of NF-κB-mediated neuronal cell apoptosis in cerebral ischemia-reperfusion injured rats. *Mol Med Rep.* (2013) 7:1516–22. doi: 10.3892/mmr.2013.1392

13. Yang Y, Hu S, Lin H, He J, Tang C. Electroacupuncture at GV24 and bilateral GB13 improves cognitive ability via influences the levels of Aβ, p-tau (s396) and p-tau (s404) in the hippocampus of Alzheimer's disease model rats. *Neuroreport.* (2020) 31:1072–83. doi: 10.1097/WNR.0000000000001518

14. Zhu M. *The Medical Classic of the Yellow Emperor.* (2001). Beijing: Foreign Languages Press.

15. Wang T. *Acupuncture for Brain: Treatment for Neurological and Psychologic Disorders.* (2021). Cham: Springer Nature.

16. Deng M, Wang X-F. Acupuncture for amnestic mild cognitive impairment: a meta-analysis of randomised controlled trials. *Acupunct Med.* (2016) 34:342–8. doi: 10.1136/acupmed-2015-010989

17. Lu X, Hongcai S, Jiaying W, Jing H, Jun X. Assessing the quality of reports about randomized controlled trials of acupuncture treatment on mild cognitive impairment. *PLoS ONE.* (2011) 6:e16922. doi: 10.1371/journal.pONE.0016922

18. Ellegaard O, Wallin JA. The bibliometric analysis of scholarly production: how great is the impact? *Scientometrics.* (2015) 105:1809–31. doi: 10.1007/s11192-015-1645-z

19. van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics.* (2010) 84:523–38. doi: 10.1007/s11192-009-0146-3

20. Chen C. CiteSpace II: Detecting and visualizing emerging trends and transient patterns in scientific literature. *J Am Soc Inform Sci Technol.* (2006) 57:724. doi: 10.1002/asi.20317

21. Chen C, Hu Z, Liu S, Tseng H. Emerging trends in regenerative medicine: a scientometric analysis in CiteSpace. *Expert Opin Biol Ther.* (2012) 12:593–608. doi: 10.1517/14712598.2012.674507

22. Chen C. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci U S A.* (2004) 101 Suppl 1:5303–10. doi: 10.1073/pnas.0307513100

23. Chen CM, Leydesdorff L. Patterns of Connections and Movements in Dual-Map Overlays: A New Method of Publication Portfolio Analysis. *J Assoc Inf Sci Technol.* (2014) 65:334–51. doi: 10.1002/asi.22968

24. PRICE DJdS. (1963). *Little science, Big science.* Columbia: Columbia University Press.

25. Pappa S, Ntella V, Giannakas T, Giannakoulis VG, Papoutsi E, Katsaounou P. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: a systematic review and meta-analysis. *Brain Behav Immun.* (2020) 88:901–7. doi: 10.1016/j.bbi.2020.05.026

26. Takeda T, Hosokawa M, Higuchi K. Senescence-accelerated mouse (SAM): a novel murine model of accelerated senescence. *J Am Geriatr Soc.* (1991) 39:911–9. doi: 10.1111/j.1532-5415.1991.tb04460.x

27. Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, et al. A new murine model of accelerated senescence. *Mech Ageing Dev.* (1981) 17:183–94. doi: 10.1016/0047-6374(81)90084-1

28. Takeda T. Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. *Neurochem Res.* (2009) 34:639–59. doi: 10.1007/s11064-009-9922-y

29. Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA, et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. *Hum Mol Genet.* (2004) 13:159–70. doi: 10.1093/hmg/ddh019

30. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci.* (2006) 26:10129–40. doi: 10.1523/JNEUROSCI.1202-06.2006

31. Colom LV, Castaneda MT, Hernandez S, Perry G, Jaime S, Touhami A. Intrahippocampal amyloid-β (1-40) injections injure medial septal neurons in rats. *Curr Alzheimer Res.* (2011) 8:832–40. doi: 10.2174/156720511798192763

32. McLarnon JG Ryu JK. Relevance of abeta1-42 intrahippocampal injection as an animal model of inflamed Alzheimer's disease brain. *Curr Alzheimer Res.* (2008) 5:475–80. doi: 10.2174/156720508785908874

33. Farkas E, Luiten PGM, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev.* (2007) 54:162–80. doi: 10.1016/j.brainresrev.2007.01.003

34. Cheng H, Yu J, Jiang Z, Zhang X, Liu C, Peng Y, et al. Acupuncture improves cognitive deficits and regulates the brain cell proliferation of SAMP8 mice. *Neurosci Lett.* (2008) 432:111–6. doi: 10.1016/j.neulet.2007.12.009

35. Li G, Zhang X, Cheng H, Shang X, Xie H, Zhang X, et al. Acupuncture improves cognitive deficits and increases neuron density of the hippocampus in middle-aged SAMP8 mice. *Acupunct Med.* (2012) 30:339–45. doi: 10.1136/acupmed-2012-010180

36. Zhao L, Zhou C, Li L, Liu J, Shi H, Kan B, et al. Acupuncture improves cerebral microenvironment in mice with Alzheimer's disease treated with hippocampal neural stem cells. *Mol Neurobiol.* (2017) 54:5120–30. doi: 10.1007/s12035-016-0054-5

37. Zhou C-L, Zhao L, Shi H-Y, Liu J-W, Shi J-W, Kan B-H, et al. Combined acupuncture and treatment affects behavior and synaptophysin levels in the hippocampus of senescence-accelerated mouse prONE 8 after neural stem cell transplantation. *Neural Regen Res.* (2018) 13:541–8. doi: 10.4103/1673-5374.228760

38. Chang S, Guo X, Li G, Zhang X, Li J, Jia Y, et al. Acupuncture promotes expression of Hsp84/86 and delays brain ageing in SAMP8 mice. *Acupunct Med.* (2019) 37:340–7. doi: 10.1136/acupmed-2017-011577

39. Zhao L, Liu J-W, Kan B-H, Shi H-Y, Yang L-P, Liu X-Y. Acupuncture accelerates neural regeneration and synaptophysin production after neural stem cells transplantation in mice. *World J Stem Cells.* (2020) 12:1576–90. doi: 10.4252/wjsc.v12.i12.1576

40. Li G, Zeng L, Cheng H, Han J, Zhang X, Xie H. Acupuncture administration improves cognitive functions and alleviates inflammation and nuclear damage by regulating phosphatidylinositol 3 kinase (PI3K)/phosphoinositol-dependent kinase 1 (PDK1)/novel protein kinase C (nPKC)/Rac 1 signaling pathway in senescence-accelerated PrONE 8 (SAM-P8) Mice. *Med Sci Monit.* (2019) 25:4082–93. doi: 10.12659/MSM.913858

41. Dong W, Guo W, Zheng X, Wang F, Chen Y, Zhang W, et al. Electroacupuncture improves cognitive deficits associated with AMPK activation in SAMP8 mice. *Metab Brain Dis.* (2015) 30:777–84. doi: 10.1007/s11011-014-9641-1

42. Dong W, Quo W, Wang F, Li C, Xie Y, Zheng X, et al. Electroacupuncture upregulates SIRT1-dependent PGC-1α expression in SAMP8 mice. *Med Sci Monit.* (2015) 21:3356–62. doi: 10.12659/MSM.894864

43. Dong W-G, Wang F, Chen Y, Zheng X-H, Xie Y-C, Guo W-Q, et al. Electroacupuncture reduces Aβ production and BACE1 expression in SAMP8 mice. *Front Aging Neurosci.* (2015) 7:148. doi: 10.3389/fnagi.2015.00148

44. Ding N, Jiang J, Xu A, Tang Y, Li Z. Manual acupuncture regulates behavior and cerebral blood flow in the SAMP8 mouse model of Alzheimer's disease. *Front Neurosci.* (2019) 13:37. doi: 10.3389/fnins.2019.00037

45. Jiang J, Liu H, Wang Z, Tian H, Wang S, Yang J, et al. Electroacupuncture could balance the gut microbiota and improve the learning and memory abilities of Alzheimer's disease animal model. *PLoS ONE.* (2021) 16:e0259530. doi: 10.1371/journal.pONE.0259530

46. Liang P-Z, Li L, Zhang Y-N, Shen Y, Zhang L-L, Zhou J, et al. Electroacupuncture improves clearance of amyloid- through the glymphatic system in the SAMP8 mouse model of Alzheimer's disease. *Neural Plast.* (2021) 2021:9960304. doi: 10.1155/2021/9960304

47. Jiang J, Liu G, Shi S, Li Y, Li Z. Effects of manual acupuncture combined with dONEpezil in a mouse model of Alzheimer's disease. *Acupunct Med.* (2019) 37:64–71. doi: 10.1136/acupmed-2016-011310

48. Dong W, Yang W, Li F, Guo W, Qian C, Wang F, et al. Electroacupuncture improves synaptic function in SAMP8 mice probably via inhibition of the AMPK/eEF2K/eEF2 signaling pathway. *Evid Based Complement Alternat Med.* (2019) 2019:8260815. doi: 10.1155/2019/8260815

49. Hou Z, Qiu R, Wei Q, Liu Y, Wang M, Mei T, et al. Electroacupuncture improves cognitive function in senescence-accelerated P8 (SAMP8) mice via the NLRP3/caspase-1 pathway. *Neural Plast.* (2020) 2020:8853720. doi: 10.1155/2020/8853720

50. Wang X, Li Z, Li C, Wang Y, Yu S, Ren L. Electroacupuncture with Bushen Jiannao improves cognitive deficits in senescence-accelerated mouse prONE 8 mice by inhibiting neuroinflammation. *J Tradit Chin Med.* (2020) 40:812–9. doi: 10.19852/j.cnki.jtcm.2020.05.011

51. Li X, Guo F, Zhang Q, Huo T, Liu L, Wei H, et al. Electroacupuncture decreases cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice. *BMC Complement Altern Med.* (2014) 14:37. doi: 10.1186/1472-6882-14-37

52. Lin R, Li L, Zhang Y, Huang S, Chen S, Shi J, et al. Electroacupuncture ameliorate learning and memory by improving N-acetylaspartate and glutamate metabolism in APP/PS1 mice. *Biol Res.* (2018) 51:21. doi: 10.1186/s40659-018-0166-7

53. Liu W, Zhuo P, Li L, Jin H, Lin B, Zhang Y, et al. Activation of brain glucose metabolism ameliorating cognitive impairment in APP/PS1 transgenic mice by electroacupuncture. *Free Radic Biol Med.* (2017) 112:174–90. doi: 10.1016/j.freeradbiomed.2017.07.024

54. Wang F, Zhong H, Li X, Peng Y, Kinden R, Liang W, et al. Electroacupuncture attenuates reference memory impairment associated with astrocytic NDRG2 suppression in APP/PS1 transgenic mice. *Mol Neurobiol.* (2014) 50:305–13. doi: 10.1007/s12035-013-8609-1

55. Lin R, Chen J, Li X, Mao J, Wu Y, Zhuo P, et al. Electroacupuncture at the Baihui acupoint alleviates cognitive impairment and exerts neuroprotective effects by modulating the expression and processing of brain-derived neurotrophic factor in APP/PS1 transgenic mice. *Mol Med Rep.* (2016) 13:1611–7. doi: 10.3892/mmr.2015.4751

56. Cao J, Tang Y, Li Y, Gao K, Shi X, Li Z. Behavioral changes and hippocampus glucose metabolism in APP/PS1 transgenic mice via electro-acupuncture at governor vessel acupoints. *Front Aging Neurosci.* (2017) 9:5. doi: 10.3389/fnagi.2017.00005

57. Xu A, Tang Y, Zeng Q, Wang X, Tian H, Zhou Y, et al. Electroacupuncture enhances cognition by promoting brain glucose metabolism and inhibiting inflammation in the APP/PS1 mouse model of Alzheimer's disease: a pilot study. *J Alzheimers Dis.* (2020) 77:387–400. doi: 10.3233/JAD-200242

58. Tang Y, Shao S, Guo Y, Zhou Y, Cao J, Xu A, et al. Electroacupuncture mitigates hippocampal cognitive impairments by reducing BACE1 deposition and activating PKA in APP/PS1 double transgenic mice. *Neural Plast.* (2019) 2019:2823679. doi: 10.1155/2019/2823679

59. Tang Y, Xu A, Shao S, Zhou Y, Xiong B, Li Z. Electroacupuncture ameliorates cognitive impairment by inhibiting the JNK signaling pathway in a mouse model of Alzheimer's disease. *Front Aging Neurosci.* (2020) 12:23. doi: 10.3389/fnagi.2020.00023

60. Xu A, Zeng Q, Tang Y, Wang X, Yuan X, Zhou Y, et al. Electroacupuncture protects cognition by regulating tau phosphorylation and glucose metabolism via the AKT/GSK3β signaling pathway in Alzheimer's disease model mice. *Front Neurosci.* (2020) 14:585476. doi: 10.3389/fnins.2020.585476

61. Yang Q, Zhu S, Xu J, Tang C, Wu K, Wu Y, et al. Effect of the electro-acupuncture on senile plaques and its formation in APP/PS1 double transgenic mice. *Genes Dis.* (2019) 6:282–9. doi: 10.1016/j.gendis.2018.06.002

62. Li J, Zhang B, Jia W, Yang M, Zhang Y, Zhang J, et al. Activation of adenosine monophosphate-activated protein kinase drives the aerobic glycolysis in hippocampus for delaying cognitive decline following electroacupuncture treatment in APP/PS1 mice. *Front Cell Neurosci.* (2021) 15:774569. doi: 10.3389/fncel.2021.774569

63. Cai M, Yang EJ. Effect of Combined Electroacupuncture and selegiline treatment in Alzheimer's disease: an animal model. *Front Pharmacol.* (2020) 11:606480. doi: 10.3389/fphar.2020.606480

64. Cai M, Lee J-H, Yang EJ. Electroacupuncture attenuates cognition impairment via anti-neuroinflammation in an Alzheimer's disease animal model. *J Neuroinflammation.* (2019) 16:264. doi: 10.1186/s12974-019-1665-3

65. Zheng X, Lin W, Jiang Y, Lu K, Wei W, Huo Q, et al. Electroacupuncture ameliorates beta-amyloid pathology and cognitive impairment in Alzheimer disease via a novel mechanism involving activation of TFEB (transcription factor EB). *Autophagy.* (2021) 17:3833–47. doi: 10.1080/15548627.2021.1886720

66. Li L, Li J, Dai Y, Yang M, Liang S, Wang Z, et al. Electro-acupuncture improve the early pattern separation in Alzheimer's disease mice basal forebrain-hippocampus cholinergic neural circuit. *Front Aging Neurosci.* (2021) 13:770948. doi: 10.3389/fnagi.2021.770948

67. Zhang M, Xv G-H, Wang W-X, Meng D-J, Ji Y. Electroacupuncture improves cognitive deficits and activates PPAR-γ in a rat model of Alzheimer's disease. *Acupunct Med.* (2017) 35:44–51. doi: 10.1136/acupmed-2015-010972

68. Guo H-D, Tian J-X, Zhu J, Li L, Sun K, Shao S-J, et al. Electroacupuncture suppressed neuronal apoptosis and improved cognitive impairment in the AD model rats possibly via downregulation of notch signaling pathway. *Evid Based Complement Alternat Med.* (2015) 2015:393569. doi: 10.1155/2015/393569

69. Yu C-C, Wang Y, Shen F, Kong L-H, Wang Y-W, Zhou H, et al. High-frequency (50 Hz) electroacupuncture ameliorates cognitive impairment in rats with amyloid beta 1-42-induced Alzheimer's disease. *Neural Regen Res.* (2018) 13:1833–41. doi: 10.4103/1673-5374.238620

70. Wang Y, Zheng A, Yang H, Wang Q, Ren B, Guo T, et al. "Olfactory three-needle" acupuncture enhances synaptic function in Aβ-induced Alzheimer's disease via activating PI3K/AKT/GSK-3β signaling pathway. *J Integr Neurosci.* (2021) 20:55–65. doi: 10.31083/j.jin.2021.01.224

71. Wang X-R, Shi G-X, Yang J-W, Yan C-Q, Lin L-T, Du S-Q, et al. Acupuncture ameliorates cognitive impairment and hippocampus neuronal loss in experimental vascular dementia through Nrf2-mediated antioxidant response. *Free Radic Biol Med.* (2015) 89:1077–84. doi: 10.1016/j.freeradbiomed.2015.10.426

72. Zhu W, Wang X-R, Du S-Q, Yan C-Q, Yang N-N, Lin L-L, et al. Anti-oxidative and Anti-apoptotic Effects of Acupuncture: Role of Thioredoxin-1 in the Hippocampus of Vascular Dementia Rats. *Neuroscience.* (2018) 379:281–91. doi: 10.1016/j.neuroscience.2018.03.029

73. Du S-Q, Wang X-R, Zhu W, Ye Y, Yang J-W, Ma S-M, et al. Acupuncture inhibits TXNIP-associated oxidative stress and inflammation to attenuate cognitive impairment in vascular dementia rats. *CNS Neurosci Ther.* (2018) 24:39–46. doi: 10.1111/cns.12773

74. Qiu J, Shen B, Zhao M, Wang Z, Xie B, Xu Y, et al. nationwide survey of psychological distress among Chinese people in the COVID-19 epidemic: implications and policy recommendations. *Gen Psychiatr.* (2020) 33:e100213. doi: 10.1136/gpsych-2020-100213

75. Ye Y, Li H, Yang J-W, Wang X-R, Shi G-X, Yan C-Q, et al. Acupuncture attenuated vascular dementia-induced hippocampal long-term potentiation impairments via activation of D1/D5 receptors. *Stroke.* (2017) 48:1044–51. doi: 10.1161/STROKEAHA.116.014696

76. Li H, Liu Y, Lin L-T, Wang X-R, Du S-Q, Yan C-Q, et al. Acupuncture reversed hippocampal mitochondrial dysfunction in vascular dementia rats. *Neurochem Int.* (2016) 92:35–42. doi: 10.1016/j.neuint.2015.12.001

77. Kang L, Li Y, Hu S, Chen M, Yang C, Yang BX, et al. The mental health of medical workers in Wuhan, China dealing with the 2019 novel coronavirus. *Lancet Psychiatry.* (2020) 7:e14. doi: 10.1016/S2215-0366(20)30047-X

78. Yang J-W, Wang X-R, Zhang M, Xiao L-Y, Zhu W, Ji C-S, et al. Acupuncture as a multifunctional neuroprotective therapy ameliorates cognitive impairment in a rat model of vascular dementia: a quantitative iTRAQ proteomics study. *CNS Neurosci Ther.* (2018) 24:1264–74. doi: 10.1111/cns.13063

79. Zhu Y, Zeng Y, Wang X, Ye X. Effect of electroacupuncture on the expression of mTOR and eIF4E in hippocampus of rats with vascular dementia. *Neurol Sci.* (2013) 34:1093–7. doi: 10.1007/s10072-012-1209-4

80. Zhu Y, Wu Q, Lin L. Effects of electro-acupuncture on Noxa and caspase-3 expression in hippocampal CA1 region of a vascular dementia rat model. *Neural Regener Res.* (2008) 3:826–31.

81. Zhu Y, Zeng Y. Electroacupuncture protected pyramidal cells in hippocampal CA1 region of vascular dementia rats by inhibiting the expression of p53 and Noxa. *CNS Neurosci Ther.* (2011) 17:599–604. doi: 10.1111/j.1755-5949.2010.00192.x

82. Wei D, Jia X, Yin X, Jiang W. Effects of electroacupuncture versus nimodipine on long-term potentiation and synaptophysin expression in a rat model of vascular dementia. *Neural Regener Res.* (2011) 6:2357–61. doi: 10.3969/j.issn.1673-5374.2011.30.007

83. Bu Y, Li W-S, Lin J, Wei Y-W, Sun Q-Y, Zhu S-J, et al. Electroacupuncture attenuates immune-inflammatory response in hippocampus of rats with vascular dementia by inhibiting TLR4/MyD88 signaling pathway. *Chin J Integr Med.* (2022) 28:153–61. doi: 10.1007/s11655-021-3350-5

84. Zhu Y, Wang X, Ye X, Gao C, Wang W. Effects of electroacupuncture on the expression of p70 ribosomal protein S6 kinase and ribosomal protein S6 in the hippocampus of rats with vascular dementia. *Neural Regener Res.* (2012) 7:207–11. doi: 10.3969/j.issn.1673-5374.2012.03.009

85. Xiao Q, Yan P, Ma X, Liu H, Perez R, Zhu A, et al. Neuronal-targeted TFEB accelerates lysosomal degradation of APP, reducing Aβ generation and amyloid plaque pathogenesis. *J Neurosci.* (2015) 35:12137–51. doi: 10.1523/JNEUROSCI.0705-15.2015

86. Feng Y, Bai L, Ren Y, Chen S, Wang H, Zhang W, et al. FMRI connectivity analysis of acupuncture effects on the whole brain network in mild cognitive impairment patients. *Magn Reson Imaging.* (2012) 30:672–82. doi: 10.1016/j.mri.2012.01.003

87. Zheng W, Su Z, Liu X, Zhang H, Han Y, Song H, et al. Modulation of functional activity and connectivity by acupuncture in patients with Alzheimer disease as measured by resting-state fMRI. *PLoS ONE.* (2018) 13:e0196933. doi: 10.1371/journal.pONE.0196933

88. Chen S, Bai L, Xu M, Wang F, Yin L, Peng X, et al. Multivariate granger causality analysis of acupuncture effects in mild cognitive impairment patients: an FMRI study. *Evid Based Complement Alternat Med.* (2013) 2013:127271. doi: 10.1155/2013/127271

89. Yan W-T, Yang Y-D, Hu X-M, Ning W-Y, Liao L-S, Lu S, et al. Do pyroptosis, apoptosis, and necroptosis (PANoptosis) exist in cerebral ischemia? Evidence from cell and rodent studies. *Neural Regen Res.* (2022) 17:1761–8. doi: 10.4103/1673-5374.331539

90. Chen Y, Li Y, Guo L, Hong J, Zhao W, Hu X, et al. Bibliometric analysis of the inflammasome and pyroptosis in brain. *Front Pharmacol.* (2020) 11:626502. doi: 10.3389/fphar.2020.626502

91. Yan W-T, Lu S, Yang Y-D, Ning W-Y, Cai Y, Hu X-M, et al. Research trends, hot spots and prospects for necroptosis in the field of neuroscience. *Neural Regen Res.* (2021) 16:1628–37. doi: 10.4103/1673-5374.303032

frontiers | Frontiers in Neurology

TYPE Systematic Review
PUBLISHED 13 October 2022
DOI 10.3389/fneur.2022.1005819

OPEN ACCESS

EDITED BY
Jingling Chang,
Dongzhimen Hospital, Beijing University of Chinese Medicine, China

REVIEWED BY
Haijun Zhao,
Shandong University of Traditional Chinese Medicine, China
Jian Pei,
Shanghai University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Xiao Liang
liangxiao022@126.com
Yunling Zhang
yunlingzhang2004@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 28 July 2022
ACCEPTED 20 September 2022
PUBLISHED 13 October 2022

CITATION
Wang L, Chi X, Lyu J, Xu Z, Fu G, Liu Y, Liu S, Qiu W, Liu H, Liang X and Zhang Y (2022) An overview of the evidence to guide decision-making in acupuncture therapies for early recovery after acute ischemic stroke. *Front. Neurol.* 13:1005819. doi: 10.3389/fneur.2022.1005819

An overview of the evidence to guide decision-making in acupuncture therapies for early recovery after acute ischemic stroke

Liuding Wang†, Xiansu Chi†, Jian Lyu, Zhenmin Xu, Guojing Fu, Yue Liu, Shaojiao Liu, Wenran Qiu, Hongxi Liu, Xiao Liang* and Yunling Zhang*

Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, China

Background: Acupuncture is a proven technique of traditional Chinese medicine (TCM) for ischemic stroke. The purpose of this overview was to summarize and evaluate the evidence from current systematic reviews (SRs) of acupuncture for early recovery after acute ischemic stroke (AIS).

Methods: We performed a comprehensive search for SRs of acupuncture for AIS in seven electronic databases up to May 23, 2022. Two reviewers independently selected SRs, extracted data, evaluated the methodological quality using the Assessment of Multiple Systematic Reviews 2 (AMSTAR 2), and rated evidence certainty using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE).

Results: Seven SRs were included. The overall methodological quality of SRs was critically low. As for GRADE, 3 outcomes had moderate-quality evidence, 14 had low-quality evidence, and 12 had very low-quality evidence. Moderate-quality evidence demonstrated that initiating acupuncture therapies within 30 days of AIS onset significantly improves neurological function and the total effective rate of patients. Low-quality evidence showed that for patients within 2 weeks of AIS onset Xingnao Kaiqiao acupuncture (XNKQ Ac) could reduce disability rate and might reduce mortality. Regarding the safety of acupuncture therapies, low-quality evidence showed that there was no difference in the incidence of adverse reactions between the 2 groups, and very-low quality evidence showed that acupuncture did not promote hemorrhagic conversion.

Conclusions: In the acute and early recovery phases after AIS onset, acupuncture is a promising therapeutic strategy to improve the curative effect of current treatments, especially in the recovery of neurological function. Patients in the acute phase might receive XNKQ Ac, and patients in the early recovery phase might receive EA[1], CA, or SA. However, considering the current certainty of evidence, a solid recommendation warrants further exploration.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022335426.

KEYWORDS

acupuncture, acute ischemic stroke, neurological function, GRADE, AMSTAR-2

Introduction

At present, acute ischemic stroke (AIS) remains a prominent cause of death and disability worldwide (1), despite breakthroughs in emergency therapy over the past years. Intravenous thrombolysis (IVT) and endovascular therapies (EVTs) have been the preferred treatments for patients with AIS (2). However, the majority of AIS patients did not receive IVT or EVTs due to late arrival to emergency departments. As for patients fortunately treated with recanalization therapies, they may suffer from ischemia/reperfusion (I/R) injury caused by highly harmful oxidative stress (OS) (3). In other words, even with currently evidence-based, effective therapies, there is a lack of an optimum therapeutic strategy to timely protect the brain from damage in the acute or early recovery stages.

As one of the various modalities of traditional Chinese medicine (TCM), acupuncture has gained international recognition, particularly in recent years (4). In the treatment of ischemic stroke, previous studies have indicated that acupuncture might prevent secondary brain injury by reducing oxidation (5). This potential mechanism of acupuncture removing superoxide has also been demonstrated in other nervous system diseases, such as vascular dementia and spinal cord injury (6–8). In animal models of ischemic stroke, acupuncture therapies may not only suppress the excessive production of reactive oxygen species (ROS), but also activate the inherent antioxidant enzymes (9). Fundamentally, acupuncture therapies may ameliorate mitochondrial dysfunction, which is manifested in raising the activities of mitochondrial respiratory enzymes (10). Regarding clinical benefits, there were numerous systematic reviews (SRs) evaluating the efficacy of acupuncture therapies for ischemic stroke from acute to convalescent and sequela stages (11–14). Since oxidative damage is the most severe within 24 h after onset (15), it is of great significance to investigate the efficacy and safety of acupuncture for ischemic stroke during the acute stage. A network meta-analysis also showed that the optimal time-point of acupuncture for stroke was within 48 h post-stroke, and the vital validity period lasted until 15 days after the attack (16). However, the paucity of systematic evaluation of evidence certainty is the reason why acupuncture therapies cannot be brought to the bedside of patients with AIS. The overview of SRs has been generally recognized to facilitate clinical decision-making. A study published in the *British Medical Journal* strongly calls for more effective evidence dissemination of acupuncture to solve the dilemma that evidence on acupuncture is underused in clinical practice (4). Therefore, we conducted this overview to summarize the existing evidence and critically evaluate the overall evidence quality of acupuncture therapies for early recovery after AIS.

Methods

This overview was carried out according to the Cochrane Handbook for SRs of Interventions (17) and registered under the number CRD42022335426.

Inclusion and exclusion criteria

We included SRs of randomized controlled trials (RCTs), in which participants were diagnosed with AIS and in the acute and early recovery phases (within 1 month after AIS onset). Acupuncture therapies were used alone or combined with conventional therapy (CT, including recanalization treatments, controlling vascular risk factors, improving blood circulation, and protecting brain cells). Comparator interventions were CT alone, or CT combined with placebo or sham acupuncture. Outcomes included but were not limited to functional independence (modified Rankin Scale score 0–2), mortality, disability rate, neurologic deficit score (NDS), activities of daily living (ADL), and adverse reactions.

We excluded studies if they were repeated publications; if their full text were unavailable; if they had incomplete or inaccurate data; if they used other TCM treatments in either intervention or control group.

Search strategy

We searched seven electronic databases listed below from their inception to 23 May 2022: MEDLINE Ovid (1946 to 23 May 2022), EMBASE Ovid (1996 to 2022 Week 20), the Cochrane Library, Chinese Biomedical Literature Service System (SinoMed), China National Knowledge Infrastructure (CNKI), Chinese Scientific Journals Database (VIP), and WanFang database. The search strategies for all databases are shown in Supplementary Table 1.

Study selection and data extraction

Two independent authors (LDW and ZMX) screened the records yielded in searches by reading titles and abstracts. Full texts of preliminary included SRs were further checked, and finally, eligible SRs were identified. And then they used a standard form extracting the following data: (1) first author, country, and publication year; (2) the number of trials and participants, eligibility criteria, interventions, comparisons, outcomes, and conclusions. Disagreements were resolved by consensus.

Quality assessment

Methodological quality of included reviews

Two independent reviewers (XSC and GJF) used the Assessment of Multiple Systematic Reviews 2 (AMSTAR 2) to evaluate the methodological quality of SRs (18). Each of the 16 items was evaluated as "yes," "partial yes," or "no." Items 2, 4, 7, 9, 11, and 13 were regarded as critical. The overall quality was rated as "high (no or 1 non-critical weakness)," "moderate (more than 1 non-critical weakness)," "low (1 critical flaw with or without non-critical weaknesses)," or "critically low (more than 1 critical flaw with or without non-critical weaknesses)." Disagreements were resolved by an expert in methodology (JL). We summarized these results and identified common methodological deficiencies.

Evidence certainty of included reviews

We used the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) system to rate the certainty of evidence as "high," "moderate," "low" or "very low." Certainty was downgraded for five factors (risk of bias, heterogeneity, detected publication bias, imprecision, and indirectness). If possible, we used the GRADEpro "Summary of findings" tables obtained from each included review. Two independent reviewers (WRQ and HXL) performed the evaluation. In cases of any disagreements, we consulted with an expert in methodology (JL).

Results

Literature search

A total of 600 records were retrieved. After removing 32 duplicates, we screened the titles and the abstracts of 556 studies, and then assessed the full texts of 12 studies. A list of 5 studies, that appeared to meet the eligibility criteria but were excluded, is shown in Supplementary Table 2 along with reasons for exclusion. Ultimately, 7 SRs met the eligibility criteria (19–25). Figure 1 shows details of selecting studies.

Characteristics of included reviews

Table 1 presents a summary of included SRs. Original trials were published between 1996 and 2020. The date of the last retrieval in the SRs varied between October 2007 and December 2020. Four SRs were in the English language (19–22), and the other 3 were Chinese (23–25). The number of participants varied across SRs, ranging from 429 to 3,792. In total, 114 RCTs involving 9921 participants were included in 7 SRs. The time to initiate acupuncture therapies in patients was within 48 h of AIS onset in 1 SR (23), 14 days in 2 SRs (20, 22), and 1 month in 4 SRs (19, 21, 24, 25). Acupuncture therapies included conventional acupuncture (CA), electroacupuncture (EA^1), eye acupuncture (EA^2), foot acupuncture (FA), scalp acupuncture (SA), governor vessel acupuncture (GV Ac), and Xingnao Kaiqiao needling method acupuncture (XNKQ Ac). SRs reported outcomes: mortality, disability rate, modified Rankin Scale (mRS), NDS, ADL, motor impairment, total effective rate, complete recanalization, hemorrhagic conversion, C-reaction protein (CRP), adverse reactions, and adverse events. The measurements of NDS included the National Institute of Health Stroke Scale (NIHSS), the Chinese Stroke Scale/Modified Edinburgh-Scandinavia Stroke Scale (CSS/MESSS), and the Scandinavia Stroke Scale (SSS). ADL was assessed using the Barthel Index (BI). Motor impairment was measured with the Fugl–Meyer Assessment (FMA). Most SRs concluded that acupuncture is effective in treating AIS. Nevertheless, the paucity of high-quality RCTs downgraded the certainty of evidence.

Efficacy of acupuncture for AIS

Results of all efficacy outcomes are presented in tabular form (Supplementary Table 3).

NDS

Acupuncture + conventional therapy vs. conventional therapy

Four SRs adopted NIHSS measuring NDS and revealed that acupuncture therapies were associated with a significant reduction in NIHSS (19, 21, 23, 24). The heterogeneity of 2 SRs was insignificant (4 RCTs, $MD = -1.18$, 95% CI −1.52 to −0.83, $P < 0.00001$, $I^2 = 0\%$; 17 RCTs, $MD = -1.86$, 95% CI −2.06 to −1.66, $P < 0.00001$, $I^2 = 22\%$) (19, 24). The other SRs had significant heterogeneity (6 RCTs, $SMD = -0.81$, 95% CI −1.14 to −0.49, $P < 0.00001$, $I^2 = 51\%$; 12 RCTs, $MD = -3.51$, 95% CI −4.54 to −2.48, $P < 0.00001$, $I^2 = 90\%$).

Three SRs used CSS/MESSS to assess NDS and revealed that acupuncture therapies were associated with a significant reduction in CSS/MESSS (19, 21, 22). The heterogeneity of 1 SR was insignificant (3 RCTs, $MD = -3.77$, 95% CI −4.98 to −2.57, $P < 0.00001$, $I^2 = 0\%$) (19), and that of the other SRs was significant (5 RCTs, $SMD = -1.27$, 95% CI −2.18 to −0.37, $P = 0.006$, $I^2 = 94\%$; 7 RCTs, $MD = -3.89$, 95% CI −5.36 to −2.43, $P < 0.00001$, $I^2 = 57\%$).

One SR used SSS to assess NDS and reported that SSS in the acupuncture group was higher than that in the control group with statistical significance (25), but the heterogeneity was significant (3 RCTs, $MD = 3.49$, 95% CI 2.00 to 4.99, $P < 0.00001$, $I^2 = 81.9\%$).

Governor vessel acupuncture vs. conventional acupuncture

One SR reported that NIHSS in the GV Ac group was lower than that in the CA group with statistical significance, but

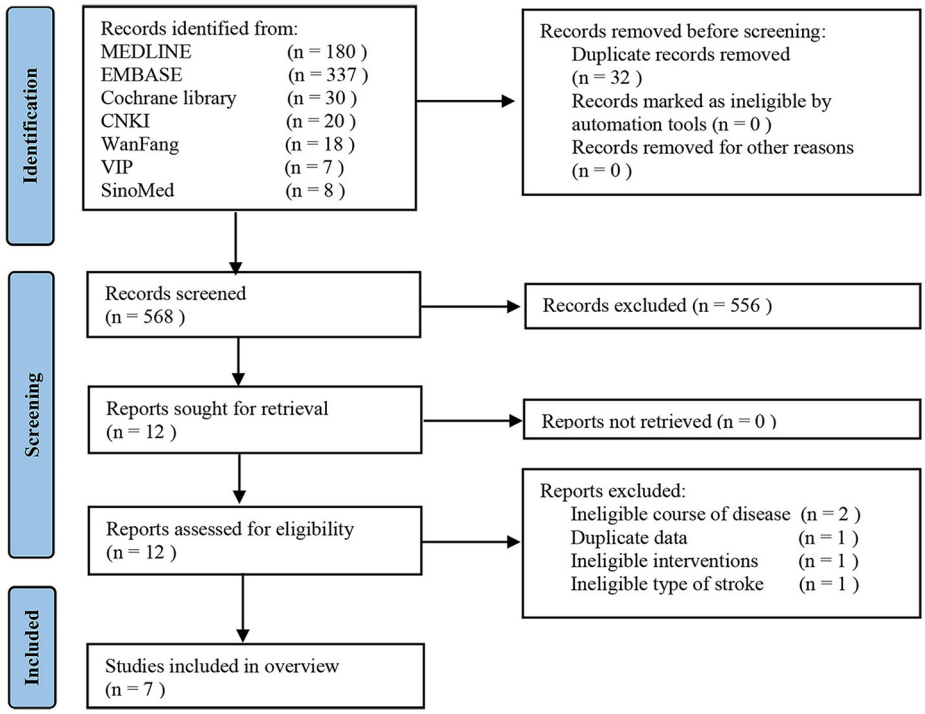


FIGURE 1
Flow diagram for identification of studies.

the heterogeneity was significant (2 RCTs, $MD = -1.32$, 95% CI −2.18 to −0.47, $P = 0.002$, $I^2 = 75\%$) (19).

One SR reported that CSS/MESSS in the GV Ac group was lower than that in the CA group with statistical significance (19), but the heterogeneity was significant (3 RCTs, $MD = -4.63$, 95% CI −5.91 to −3.35, $P < 0.00001$, $I^2 = 50\%$).

BI

Acupuncture + conventional therapy vs. conventional therapy

Three SRs showed that the acupuncture group was superior to the control group in improving BI with statistical significance. In these 3 SRs, 1 SR performed a subgroup analysis according to the course of treatment (2 RCTs, $MD_{\leq 15d} = 22.55$, 95% CI 18.66 to 26.45, $P < 0.00001$, $I^2 = 0\%$; 3 RCTs, $MD_{>15d} = 8.80$, 95% CI 5.87 to 11.72, $P < 0.0001$, $I^2 = 0\%$) (19). A subgroup analysis according to thrombolytic drugs was performed in another SR (23), which failed to explain the significant heterogeneity.

Governor vessel acupuncture vs. conventional acupuncture

One SR reported that BI in the GV Ac group was higher than that in the CA group with statistical significance (19), but the heterogeneity was significant (5 RCTs, $MD = 8.27$, 95% CI 4.29 to 12.26, $P < 0.0001$, $I^2 = 78\%$).

Xingnao kaiqiao needling method acupuncture + conventional acupuncture vs. conventional acupuncture

One SR reported that XNKQ Ac plus CA improved BI better than CA alone (1 RCT, $MD = 17.17$, 95% CI 9.15 to 25.19, $P < 0.0001$) (20).

mRS

Acupuncture + conventional therapy vs. conventional therapy

One SR reported that mRS at 1 month in the acupuncture group was lower than that in the control group (3 RCTs, $MD = -0.63$, 95% CI −0.95 to −0.32, $P < 0.0001$, $I^2 = 0\%$) (19). Although the difference had statistical significance, the effect size was far <1 point. As for the evaluation of mRS, 1 point is required per grade. In terms of clinical benefits, therefore, the clinical significance of the difference was insignificant.

FMA

Acupuncture + conventional therapy vs. conventional therapy

One SR reported that FMA in the acupuncture group was higher than that in the control group with statistical significance (8 RCTs, $SMD = 0.98$, 95% CI 0.75 to 1.22, $P < 0.00001$, $I^2 = 36\%$) (21).

TABLE 1 Characteristics of included SRs.

Reference	Language	No. of studies (participants)	Course of disease	Treatment intervention vs. control intervention	Quality assessment tool	Outcome (s)	Main conclusion
Shao et al. (19)	English	18 (1,543)	<30 days	GV Ac + CT vs. CT; GV Ac + CT vs. CA + CT	the Cochrane risk of bias tool	Neurological deficit score, activities of daily living, functional independence, adverse events	GV Ac combined with CT may increase the benefit, and for efficacy GV Ac appears to be better than that of ordinary acupuncture.
Yang et al. (20)	English	12 (1,006)	<14 days	XNKQ Ac + CT vs. CT; XNKQ Ac + CA vs. CA	the Cochrane risk of bias tool	Mortality, disability rate, activities of daily living, total effective rate, adverse events	XNKQ Ac might significantly reduce the disability rate, but it had no significant impact on mortality. XNKQ Ac is effective and safe for AIS, but more high-quality randomized controlled trials are needed to provide reliable evidence.
Liu et al. (21)	English	18 (1,411)	<30 days	EA[1] + CT vs. CT	the Cochrane risk of bias tool	Neurological deficit score, activities of daily living, motor function, total effective rate, adverse events	EA was effective and generally safe for AIS, although further larger sample-size and rigorously designed RCTs are required.
Wang et al. (22)	English	8 (538)	<14 days	SA + CT vs. CT	the Cochrane risk of bias tool	Neurological deficit score, total effective rate, adverse events	SA appears to be able to improve neurological deficit score and the clinical effective rate, though the beneficial effect from SA is possibly overvalued because of generally low methodology of the included trials. Rigorous well-designed clinical trials are needed.
Zhang et al. (23)	Chinese	14 (1,202)	≤48 h	XNKQ Ac, EA[2], FA, or CA + thrombolysis + CT vs. thrombolysis + CT	the Cochrane risk of bias tool	Neurological deficit score, activities of daily living, total effective rate, C-reaction protein level, the rate of complete recanalization, hemorrhagic conversions, incidence of adverse reactions	Acupuncture has certain advantages in improving the therapeutic effect and safety of thrombolysis in the treatment of AIS.
Zhang and Li (24)	Chinese	39 (3,792)	≤30 days	CA, EA[1], EA[2], SA, XNKQ Ac, or A&M + CT vs. CT	NOS scale	Neurological deficit score, total effective rate	Acupuncture plus CT is effective in treating AIS, but more randomized, double-blind, large-sample trials are needed.
Zhang et al. (25)	Chinese	5 (429)	<28 days	CA or EA[1] + CT vs. CT	/	Mortality, disability rate, neurological deficit score	The result cannot prove that acupuncture can set down disability and mortality. Although acupuncture can improve the neurological function of AIS patients, we cannot sure the curative effect. We need randomized, double blinded, controlled trials with high-quality, large-sample, multi-center to get believable evidence.

Ac, acupuncture; A&M, acupuncture and moxibustion; CA, conventional acupuncture; CT, conventional therapy; EA[1], electroacupuncture; EA[2], eye acupuncture; FA, foot acupuncture; GV, governor vessel; SA, scalp acupuncture; XNKQ, Xingnao Kaiqiao needling method.

10.3389/fneur.2022.1005819

Mortality and disability rate

Xingnao kaiqiao acupuncture + conventional acupuncture vs. conventional acupuncture

After 3 or 6 months of follow-up, 1 SR reported that XNKQ Ac plus CA might have additional effects in reducing disability rate with statistical significance and mortality without statistical significance (3 RCTs, $RR_{disability} = 0.51$, 95% *CI* 0.27 to 0.98, $P = 0.04$, $I^2 = 0\%$; 3 RCTs, $RR_{mortality} = 0.58$, 95% *CI* 0.17 to 1.93, $P = 0.37$, $I^2 = 0\%$) (20). The confidence interval of disability rate was so wide that it was close to the invalid line, and the confidence interval of mortality even included the invalid line. Given the imprecision, the reliability of the results is questionable.

Acupuncture + conventional therapy vs. conventional therapy

One SR defined disabled as BI $\leq$ 60 (25), and reported that there was no statistical difference in the rate of death or disability at 6 months of follow-up between the acupuncture group and the control group. However, there was a trend toward reduced rate of death or disability with additional acupuncture therapies (3 RCTs, $OR = 0.59$, 95% *CI* 0.31 to 1.13, $P = 0.11$, $I^2 = 0\%$).

Total effective rate

Acupuncture + conventional therapy vs. conventional therapy

Four SRs reported that acupuncture might improve total effective rate with insignificant heterogeneity (6 RCTs, $RR = 1.42$, 95% *CI* 1.18 to 1.72, $P = 0.0002$, $I^2 = 16\%$; 4 RCT, $RR = 1.23$, 95% *CI* 1.11 to 1.37, $P < 0.01$, $I^2 = 0\%$; 13RCTs, $RR = 1.19$, 95% *CI* 1.13 to 1.25, $P < 0.00001$, $I^2 = 41\%$; 26 RCTs, $OR = 3.95$, 95% *CI* 3.02 to 5.16, $P < 0.00001$, $I^2 = 0\%$) (21–24). Another SR performed a subgroup analysis of different courses of disease (2 RCTs, $RR_{\leq 24h} = 1.40$, 95% *CI* 1.06 to 1.86, $P = 0.02$, $I^2 = 2\%$; 2 RCT, $RR_{6h-72h} = 1.63$, 95% *CI* 1.03 to 2.59, $P = 0.04$, $I^2 = 45\%$) (20).

Xingnao kaiqiao acupuncture + conventional acupuncture vs. conventional acupuncture

One SR reported that the total effective rate in the XNKQ Ac plus CA group was higher than that in the CA group without statistical significance (1 RCT, $RR = 1.80$, 95% *CI* 1.00 to 3.23, $P = 0.05$) (20).

Rate of complete recanalization

Acupuncture + conventional therapy vs. conventional therapy

One SR found that the use of acupuncture after thrombolysis might improve the rate of complete recanalization without statistical significance (2 RCTs, $RR = 1.20$, 95% *CI* 1.00–1.44, $P = 0.05$, $I^2 = 0\%$) (23).

TABLE 2 Methodological quality assessment by AMSTAR 2.

Reviews	AMSTAR-2															Total yes	Overall quality
	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14	Item 15	Item 16		
Shao et al. (19)	Y	Y	N	Y	Y	Y	N	PY	Y	N	Y	N	N	Y	Y	Y	10	Critically low
Yang et al. (20)	N	N	N	Y	Y	Y	N	Y	Y	N	Y	N	N	Y	N	Y	9	Critically low
Liu et al. (21)	Y	N	N	Y	Y	Y	N	Y	Y	N	N	Y	Y	N	Y	N	9	Critically low
Wang et al. (22)	Y	N	N	N	Y	Y	N	Y	Y	N	N	N	N	N	Y	Y	7	Critically low
Zhang et al. (23)	Y	N	N	PY	Y	Y	N	N	Y	N	Y	N	N	Y	Y	N	7	Critically low
Zhang and Li (24)	N	N	N	PY	Y	N	N	N	N	N	N	N	N	N	Y	N	2	Critically low
Zhang et al. (25)	N	N	N	Y	Y	Y	N	N	PY	N	N	N	Y	N	N	Y	5	Critically low
In total of "Y"	57.14%	14.29%	0	57.14%	100 %	85.71%	0	42.86%	71.43%	0	42.86%	14.29%	28.57%	42.86%	71.43%	57.14%		

Y, yes; PY, partial yes; N, no; Item 1, whether the research question and inclusion criteria included the components of PICO; Item 2, whether to establish the methods prior to the implementation, and whether to report significant deviations from the protocol; Item 3, whether the authors explained the selection of the study designs for inclusion; Item 4, whether the authors used a comprehensive literature search strategy; Item 5, whether the literature screening was performed in duplicate; Item 6, whether the data extraction was performed in duplicate; Item 7, whether a list of excluded studies and reasons for the exclusions were provided; Item 8, whether the authors describe the included studies in detail; Item 9, whether the authors used appropriate methods to assess the risk of bias in included studies; Item 10, whether the authors reported funding; Item 11, if meta-analysis was performed, did the authors use right methods to pool the results; Item 12, if meta-analysis was performed, did the authors consider the impact of risk of bias on the pooled results or other evidence integration; Item 13, whether the authors considered the risk of bias when interpreting the results; Item 14, whether the authors explained any heterogeneity across the review; Item 15, if quantitative synthesis was performed, did the authors detect publication bias and discuss the potential impact on the findings; Item 16, whether the authors reported any conflicts of interest.

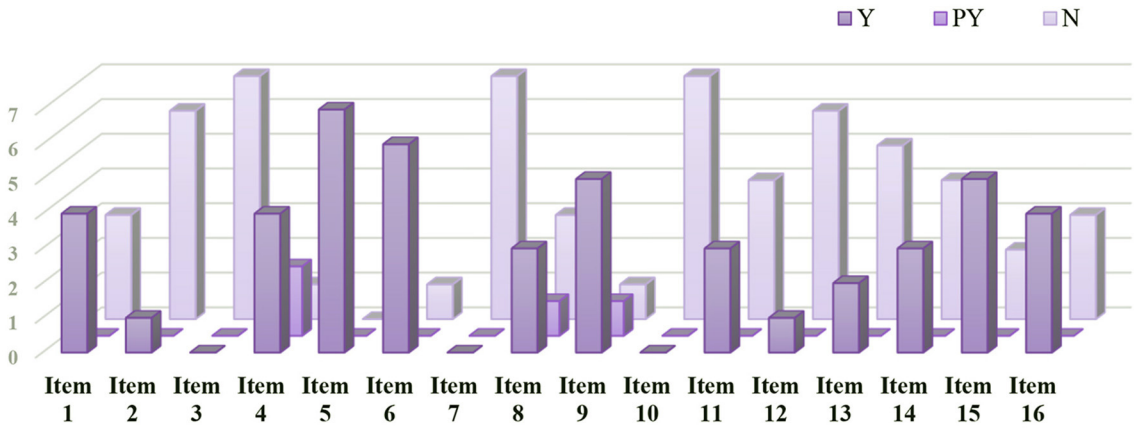


FIGURE 2
Methodological quality assessment by AMSTAR 2.

CRP

Acupuncture + conventional therapy vs. conventional therapy

One SR revealed that CRP in the acupuncture group was lower than that in the control group with statistical significance and insignificant heterogeneity (2 RCTs, $MD = -3.99$, 95% CI −4.35 to −3.63, $P < 0.00001$, $I^2 = 0\%$) (23).

Safety of acupuncture for AIS

Two SRs reported no adverse events (20, 22), and 3 SRs reported adverse events in detail (19, 21, 23). The adverse events in the acupuncture group mainly included subcutaneous hematoma, ecchymosis, and needle stagnation. One SR reported that the hemorrhagic conversion rate in the acupuncture group was lower than that in the control group without statistical significance (3 RCTs, $RR = 0.72$, 95% CI 0.14–3.62, $P = 0.69$, $I^2 = 51\%$) (23).

Quality assessment

Methodological quality of included reviews

We summarized the methodological quality of included SRs in Table 2 and Figure 2. Seven critical items were poorly reported. Just 1 SR provided a well-developed protocol (item 2) (19). Three SRs only searched electronic databases, but not clinical registries or gray literature (item 4) (22–24). None of the SRs presented a list of each excluded trial with specific reasons (item 7). Two SRs used an inappropriate technique to assess the risk of bias (RoB) (item 9) (24, 25). More than half of SRs lacked an exploration of substantial heterogeneity (item 11) (21, 22, 24, 25). And most SRs did not consider the impact of RoB when discussing their results (item 13) (19, 20, 22–24). One SR carried out a quantitative analysis, the Egger test, to detect the publication bias (19), 4 SRs performed a qualitative analysis (21–24), and 2 SRs did not mention it (item 15) (20, 25). As for 9 non-critical items, the poorly reported ones mainly were the reason for selecting RCTs (item 3), the sources of funding (item 10), and the assessment of the potential impact of RoB on meta-analysis (item 12).

Evidence certainty of included reviews

One SR evaluated the certainty of evidence (21). Considering objectivity and impartiality, we used the GRADE approach to reevaluate important outcomes of all SRs. We summarized the overall certainty of evidence in Table 3. Ratings ranged from very low to moderate. Evidence of three outcomes was moderate-quality, 14 was low-quality, and 12 was very low-quality. The main reasons for downgrading the certainty across SRs were a high risk of bias (inadequate reporting of randomization, lack of blinding) (100 %), imprecision (79.31 %), and inconsistency (48.28 %).

Subgroup analysis

Providing practical guidance in clinical decision-making, we further performed subgroup analysis. As for the NIHSS in patients within 1 month after AIS, we collected original data from 2 SRs (21, 24), pooled these results, and performed subgroup analysis according to acupuncture methods. The results demonstrated that EA[1], CA, and SA all significantly promoted early recovery of neurological function after AIS onset (Figure 3). The confidence intervals of the 3 subgroups were narrow and statistical heterogeneity was acceptable, indicating

TABLE 3 Certainty of evidence in included SRs by GRADE.

Reviews	Interventions	Outcomes	No. of RCT (participant intervention/ control group)	Certainty assessment					Overall certainty
				Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	
Shao et al. (19)	GV Ac + CT vs. CT	BI	5 (155/154)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		mRS	3 (73/74)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		NIHSS	4 (103/104)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		CSS/MESSS	3 (94/93)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
	GV Ac vs. CA	BI	5 (187/181)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		NIHSS	2 (58/57)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		CSS/MESSS	3 (127/122)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
Zhang et al. (23)	XNKQ Ac, EA[2], FA or CA + IVT vs. IVT	Total effective rate	13 (574/568)	Serious[a]	Not serious	Not serious	Not serious	Undetected	Moderate
		NIHSS	12 (509/501)	Serious[a]	Serious[b]	Not serious	Not serious	Undetected	Low
		BI	8 (345/345)	Serious[a]	Serious[b]	Not serious	Not serious	Undetected	Low
		CRP	2 (65/65)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		Incidence of adverse reaction	5 (228/228)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		Hemorrhagic conversion rate	3 (132/132)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
Zhang and Li (24)	CA, EA[1], EA[2], SA or A&M + CT vs. CT	NIHSS	17 (740/715)	Serious[a]	Not serious	Not serious	Not serious	Undetected	Moderate
		Total effective rate	26 (1,497/1,460)	Serious[a]	Not serious	Not serious	Not serious	Undetected	Moderate
Yang et al. (20)	XNKQ Ac + CT vs. CT	Disability rate	2 (63/65)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		Mortality	3 (178/182)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		BI	3 (102/104)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		Total effective rate	8 (399/402)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
	XNKQ Ac + CA vs. CA	BI	1 (30/30)	Serious[a]	-	Not serious	Serious[c]	Undetected	Very low
Liu et al. (21)	EA[1] + CT vs. CT	BI	10 (381/361)	Serious[a]	Serious[b]	Not serious	Not serious	Undetected	Low
		FMA	8 (252/247)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		NIHSS	6 (177/168)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		CSS	5 (203/194)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		Total effective rate	6 (200/195)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
Wang et al. (22)	SA + CT vs. CT	MESSS	7 (223/213)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low
		Total effective rate	4 (153/155)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
Zhang et al. (25)	CA or EA[1] + CT vs. CT	Rate of death or disability	3 (110/115)	Serious[a]	Not serious	Not serious	Serious[c]	Undetected	Low
		SSS	3 (150/135)	Serious[a]	Serious[b]	Not serious	Serious[c]	Undetected	Very low

Ac, acupuncture; A&M, acupuncture and moxibustion; CA, conventional acupuncture; CT, conventional therapy; EA[1], electroacupuncture; EA[2], eye acupuncture; FA, foot acupuncture; GV, governor vessel; IVT, intravenous thrombolysis; SA, scalp acupuncture; XNKQ, Xingnao Kaiqiao needling method. [a]High risk of bias in allocation concealment and blinding. [b]The statistical heterogeneity was significant ($I^2 > 50\%$). [c]Small sample size or too wide confidence interval.

10.3389/fneur.2022.1005819

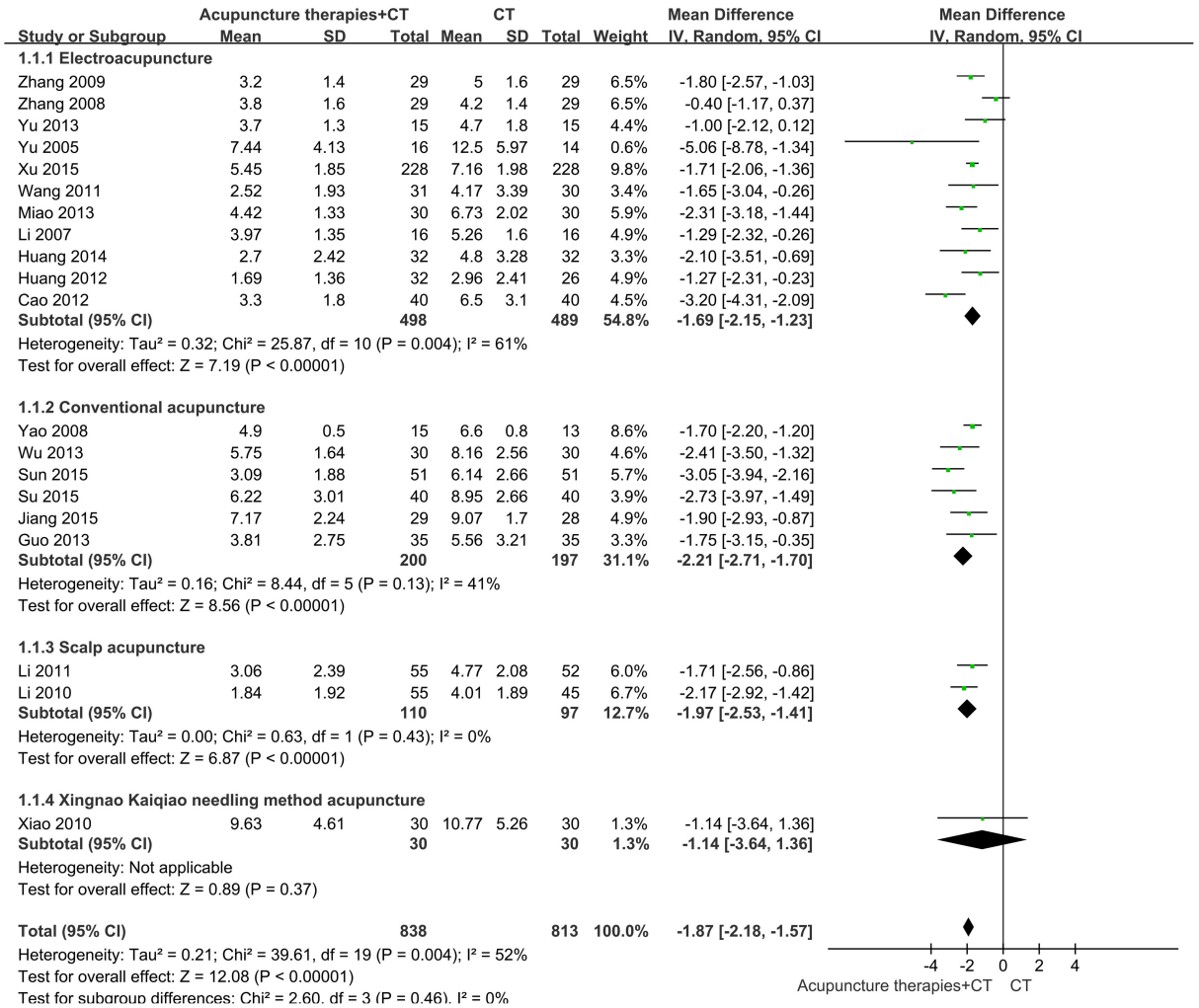


FIGURE 3
Subgroup analysis of the NIHSS in patients receiving acupuncture therapies within 1 month after AIS onset.

that the evidence was reliable. As for the NIHSS in patients within 48 hours after AIS onset, we collected original data from the SR (23) and performed subgroup analysis according to acupuncture methods. The results of all subgroups were statistically significant (Figure 4). But the larger sample size (750 participants) demonstrated that XNKQ Ac was the preferred acupuncture method for patients within 48 hours after AIS onset compared with other acupuncture methods, such as EA[2], FA, and CA.

Discussion

The SR of RCTs is ranked as the most rigorous tool for clinical decision-making. Nevertheless, the uneven quality of original RCTs and the non-standard implementation of SR affected the certainty of evidence. A growing number of SRs of acupuncture for various diseases with positive results have been published. Therefore, an overview of SRs is of great significance (26). To our knowledge, this study is the first overview of SRs regarding acupuncture for AIS. In our overview, included SRs provided invaluable evidence on acupuncture therapies for AIS treatment, but many limitations do exist.

Summary of findings

We identified 7 SRs with 114 RCTs (9,921 participants). Overall methodological quality assessed by AMSTAR 2 was low, and most critical items were reported poorly, particularly in establishing an explicit statement before the conduct of the study, listing the excluded trials with precise explanation,

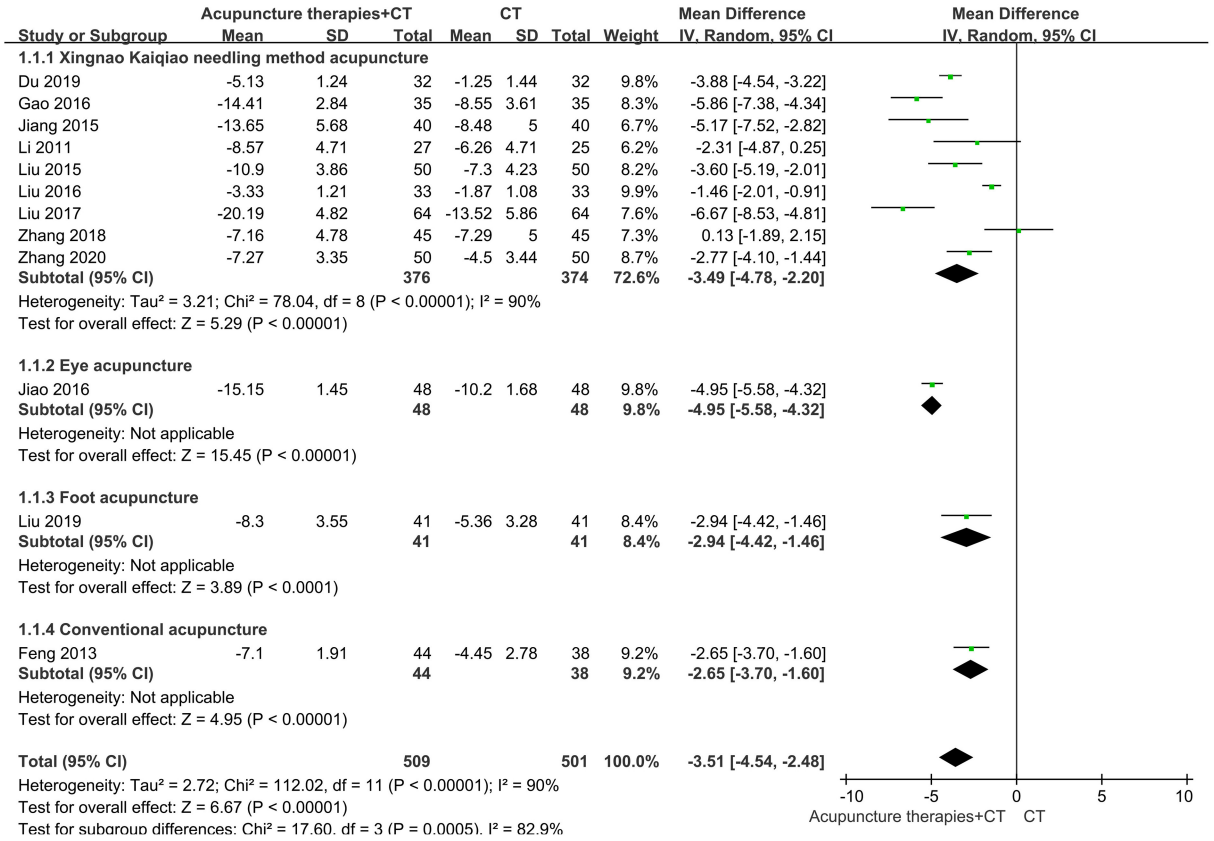


FIGURE 4
Subgroup analysis of the NIHSS in patients receiving acupuncture therapies within 48 h after AIS onset.

performing sensitivity or subgroup analyses for substantial heterogeneity, and fully assessing the impact of RoB on results. Due to the high risk of bias, significant heterogeneity, and small samples, the level of evidence was correspondingly downgraded. The certainty of evidence was moderate to very low.

Moderate-quality evidence indicated that acupuncture therapies improve neurological function and the total effective rate of patients within 30 days of AIS onset. Several experiments studying rats with middle cerebral artery occlusion demonstrated that acupuncture therapies might ameliorate neurological function by regulating the opening of large-conductance Ca2+-activated potassium channels (27), and repressing ER stress-mediated autophagy and apoptosis (28).

Low-quality evidence suggested that initiating XNKQ Ac within 2 weeks of AIS onset might reduce the disability rate and mortality. Although there was no statistical difference in mortality, the trend of significantly reducing mortality deserves more research. XNKQ Ac was developed by Professor Shi Xuemin, academician of the Chinese Academy of Engineering, in 1972. Two decades ago, the effects of inhibiting free radicals and increasing superoxide dismutase (SOD) were verified in the rabbit model of acute I/R (29). There was an RCT conducted in Germany suggesting the modulation of functional connectivity in areas of motor function by XNKQ Ac (30). A recent clinical trial, comparing XNKQ Ac plus alteplase and alteplase alone, suggested that XNKQ Ac might promote the recovery of neurological function by improving lipid peroxidation (31). Lately, an RCT conducted by Shanghai Jiao Tong University found that the effects of XNKQ Ac may be associated with modulating brain rhythm oscillations of AIS patients (32).

Low-quality evidence demonstrated that the initiation of GV Ac within 3 days of AIS onset improves mRS at 1 month. The measurement of functional independence using mRS is generally considered the primary outcome (33). However, the effect size of mRS reduced by GV Ac was only 0.63, far < 1 point. The change just had statistical significance, instead of clinical significance. Additionally, we verified that this is the mRS observed at 1 month. Confirmation of long-term efficacy also depends on whether mRS changes significantly at 3 months.

Low-quality evidence and very low-quality evidence indicated that adjuvant acupuncture after thrombolysis might

not increase the hemorrhagic conversion rate and incidence of other adverse reactions in patients within 48 h of AIS onset.

In addition to the aforementioned findings, we summarized the limitations of included SRs as follows: (1) All participants were diagnosed with AIS, but the definition of acute phase varied widely across SRs, including within 2 weeks of stroke onset, 4 weeks, 1 month, and 48 h. (2) Some SRs included 2 or more types of acupuncture, but there was no subgroup analysis based on different acupuncture therapies when performing meta-analyses. This clinical heterogeneity, to a certain extent, limited the generalizability of results.

Strengths and limitations

As the first overview of acupuncture for AIS, this study provided a series of clinical evidence, graded according to the GRADE, contributing to decision-making; revealed prevalent problems in current SRs and RCTs; and made constructive suggestions for future researchers. However, there was an inevitable limitation. The quality evaluation is subjective, although we guarantee strict adherence to internationally recognized standards.

Implications

To shorten the course of the disease, lengthen the duration of follow-up, increase sample sizes, and achieve blinding are the most critical implications for trialists. Firstly, the initiation time might be uniformly selected as the first 2 weeks after the onset of stroke. Generally, we define the acute phase as within 2 weeks (34). Moreover, the effect of acupuncture might be more significant during this period (16). Based on this premise, to further determine the optimum initiation time of acupuncture therapies, it is recommended to observe the difference in the efficacy of patients receiving acupuncture therapies within 6, 24, or 48 h of stroke onset. Secondly, given the potential advantages of acupuncture in improving disability rate and mortality after 3 or 6 months of follow-up (20), trialists should investigate endpoints, such as death, persistent disability, and recurrence of stroke, in more samples. Thirdly, trialists are suggested to blind outcome assessors, whereas sham acupuncture should be used with caution due to the underestimation of the acupuncture effect (35). As for placebo devices in acupuncture clinical trials, there was no definite evidence to support the blinding effects of these devices (36).

Reviewers of SRs should develop a protocol in advance to avoid performance bias; provide a complete list of excluded trials with exclusion reasons to avoid publication bias; fully address the heterogeneity and assess the impact of RoB on results to enhance credibility.

Conclusion

During the acute and early recovery phases after AIS onset, acupuncture therapies improve the curative effect of current treatments, especially in restoring neurological function. Based on the current evidence, it is suggested to select acupuncture methods according to the stages after AIS onset. Patients within 48 h or 2 weeks might receive XNKQ Ac, and patients within 1 month might receive EA[1], CA, or SA. Considering the certainty of evidence, trialists should verify the benefits of acupuncture therapies in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

LW, XL, and YZ designed the study. JL provided help with methodology. XL and YZ made clinical suggestions and critically revised the manuscript. LW completed the introduction section. LW and ZX conducted study selection and data extraction. XC and GF performed AMSTAR 2 assessment. WQ and HL performed a GRADE assessment. SL and YL summarized the tables. LW and XC completed the manuscript writing. ZX helped with project supervision and language revisions. All authors contributed to the article and approved the submitted version.

Funding

The study was funded by the Innovation Team and Talents Cultivation Program of the National Administration of Traditional Chinese Medicine (No. ZYYCXTD-C-202007); the project from the China Center for Evidence-Based Traditional Chinese Medicine (No. 2020YJSZX-3); the projects from China Academy of Chinese Medical Sciences (Nos. CI2021B006 and CI2021A01301); 2019 the State Administration of traditional Chinese medicine TCM evidence-based capacity building project (ZZ13-024-3) and the National TCM Leading Personnel Support Program [NATCM Personnel and Education Department (2018) No. 12].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1005819/full#supplementary-material

References

1. Campbell B, Khatri P. Stroke. *Lancet.* (2020) 396:129–42. doi: 10.1016/S0140-6736(20)31179-X

2. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American heart association/American stroke association. *Stroke.* (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211

3. Orellana-Urzúa S, Rojas I, Líbano L, Rodrigo R. Pathophysiology of ischemic stroke: role of oxidative stress. *Curr Pharm Des.* (2020) 26:4246–60. doi: 10.2174/1381612826666200708133912

4. Lu L, Zhang Y, Tang X, Ge S, Wen H, Zeng J, et al. Evidence on acupuncture therapies is underused in clinical practice and health policy. *BMJ.* (2022) 376:e67475. doi: 10.1136/bmj-2021-067475

5. Chen C, Hsieh C. Effect of acupuncture on oxidative stress induced by cerebral ischemia-reperfusion injury. *Antioxidants.* (2020) 9:248. doi: 10.3390/antiox9030248

6. Liu C, Zhou S, Guimarães S, Cho WC, Shi G. Acupuncture and oxidative stress. *Evid Based Complementary Altern Med.* (2015) 2015:1. doi: 10.1155/2015/424762

7. Zhu W, Wang X, Du S, Yan C, Yang N, Lin L, et al. Anti-oxidative and anti-apoptotic effects of acupuncture: role of thioredoxin-1 in the hippocampus of vascular dementia rats. *Neuroscience.* (2018) 379:281–91. doi: 10.1016/j.neuroscience.2018.03.029

8. Cheng M, Wu X, Wang F, Tan B, Hu J. Electro-acupuncture inhibits p66shc-mediated oxidative stress to facilitate functional recovery after spinal cord injury. *J Mol Neurosci.* (2020) 70:2031–40. doi: 10.1007/s12031-020-01609-5

9. Su X, Wang L, Ma S, Cao Y, Yang N, Lin L, et al. Mechanisms of acupuncture in the regulation of oxidative stress in treating ischemic stroke. *Oxid Med Cell Longev.* (2020) 2020:1–15. doi: 10.1155/2020/7875396

10. Zhang X, Wu B, Nie K, Jia Y, Yu J. Effects of acupuncture on declined cerebral blood flow, impaired mitochondrial respiratory function and oxidative stress in multi-infarct dementia rats. *Neurochem Int.* (2014) 65:23–9. doi: 10.1016/j.neuint.2013.12.004

11. Xu M, Li D, Zhang S. Acupuncture for acute stroke. *Cochrane Database Syst Rev.* (2018) 3:CD003317. doi: 10.1002/14651858.CD003317.pub3

12. Yang A, Wu HM, Tang JL, Xu L, Yang M, Liu GJ. Acupuncture for stroke rehabilitation. *Cochrane Database Syst Rev.* (2016) 8:CD004131. doi: 10.1002/14651858.CD004131.pub3

13. Xin Z, Xue-Ting L, De-Ying K. GRADE in systematic reviews of acupuncture for stroke rehabilitation: recommendations based on high-quality evidence. *Sci Rep.* (2015) 5:1–11. doi: 10.1038/srep16582

14. Thomas LH, Coupe J, Cross LD, Tan AL, Watkins CL. Interventions for treating urinary incontinence after stroke in adults. *Cochrane Database Syst Rev.* (2019) 2:CD004462. doi: 10.1002/14651858.CD004462.pub4

15. Žitnanová I, Šiarnik P, Kollár B, Chomová M, Pazderová P, Andrezálová L et al. Oxidative stress markers and their dynamic changes in patients after acute ischemic stroke. *Oxid Med Cell Longev.* (2016) 2016:9761697. doi: 10.1155/2016/9761697

16. Zhuo Y, Xu M, Deng S, Zhang Y, Lu X, Wu B, et al. Efficacy and safety of dissimilar acupuncture intervention time-points in treating stroke: a systematic review and network meta-analysis. *Ann Palliat Med.* (2021) 10:10196–212. doi: 10.21037/apm-21-1127

17. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. *Cochrane Handbook for Systematic Reviews of Interventions.* Chichester (UK): John Wiley & Sons (2019). doi: 10.1002/9781119536604

18. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, et al. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ.* (2017) 358:j4008. doi: 10.1136/bmj.j4008

19. Shao T, Zhou X, Ding M, Ye Z, Qian M, Jin Z. Governor vessel acupuncture for acute ischemic stroke: a systematic review and meta-analysis. *Ann Palliat Med.* (2021) 10:7236–46. doi: 10.21037/apm-21-691

20. Yang ZX, Xie JH, Liu DD. Xingnao Kaiqiao needling method for acute ischemic stroke: a meta-analysis of safety and efficacy. *Neural Regene Res.* (2017) 12:1308–14. doi: 10.4103/1673-5374.213551

21. Liu A, Li J, Li H, Fu D, Zheng G, Lu L, et al. Electroacupuncture for acute ischemic stroke: a meta-analysis of randomized controlled trials. *Am J Chin Med.* (2015) 43:1541–66. doi: 10.1142/S0192415X15500883

22. Wang Y, Fu D, Chen C, Lu L, Lu L, Xie C, et al. Scalp acupuncture for acute ischemic stroke: a meta-analysis of randomized controlled trials. *Evid Based Complement Alternat Med.* (2012) 2012:480950. doi: 10.1155/2012/480950

23. Zhang Z, Zhang X, Ni G. Thrombolysis combined with acupuncture therapy for acute cerebral infarction: a meta-analysis of randomized controlled trials. *Acupunct Res.* (2021) 46:431–8. doi: 10.13702/j.1000-0607.200559

24. Zhang S, Li Z. The effectiveness of acupuncture and moxibustion in treating acute cerebral infarction: a meta-analysis. *Chin J Phys Med Rehabil.* (2018) 40:217–22.

25. Zhang T, Zhang L, Zhang H, Li Q. Systematic review of acupuncture therapy for acute ischemic stroke. *Chin J Tradit Chin Med Pharm.* (2009) 24:101–4.

26. Hunt H, Pollock A, Campbell P, Estcourt L, Brunton G. An introduction to overviews of reviews: planning a relevant research question and objective for an overview. *Syst Rev.* (2018) 7:1–9. doi: 10.1186/s13643-018-0695-8

27. Han L, Wang Y, Wang G, Chen Y, Lin H, Zhang Y, et al. Acupuncture ameliorates neurological function in rats with cerebral ischemia-reperfusion by regulating the opening of large-conductance Ca^{2+}-activated potassium channels. *Brain Behav.* (2021) 11:e2286. doi: 10.1002/brb3.2286

28. Sun X, Liu H, Sun Z, Zhang B, Wang X, et al. Acupuncture protects against cerebral ischemia-reperfusion injury via suppressing endoplasmic reticulum stress-mediated autophagy and apoptosis. *Mol Med.* (2020) 26:105. doi: 10.1186/s10020-020-00236-5

29. Hu G, Tian F, Li P, Zhao L. Effect of Xingnao Kaiqiao acupuncture on pathological ultrastructure of brain free radicals in rabbits with cerebral ischemia and reperfusion. *Chinese Critic Care Med.* (1996) 8:5–7.

30. Nierhaus T, Chang Y, Liu B, Shi X, Yi M, Witt CM, et al. Somatosensory stimulation with XNKQ acupuncture modulates functional connectivity of motor areas. *Front Neurosci.* (2019) 13:147. doi: 10.3389/fnins.2019.00147

31. Zhang Q, Tian Z. Effects of xingnaokaiqiao acupuncture combined with alteplase on neurological deficit, lipid peroxidation and cerebrovascular reserve function in patients with acute cerebral infarction. *Shanghai J Acupunct Moxibust.* (2020) 39:25–30. doi: 10.13460/j.issn.1005-0957.2020.01.0025

32. Guo X, Zhang X, Sun M, Yu L, Qian C, Zhang J, et al. Modulation of brain rhythm oscillations by xingnao kaiqiao acupuncture correlates with stroke recovery: a randomized control trial. *J Integr Complement Med.* (2022) 28:436–4. doi: 10.1089/jicm.2021.0264

33. Broderick JP, Adeoye O, Elm J. Evolution of the modified rankin scale and its use in future stroke trials. *Stroke.* (2017) 48:2007–12. doi: 10.1161/STROKEAHA.117.017866

34. Chinese Society of Neurology, Chinese Stroke Society. Chinese guidelines for diagnosis and treatment of acute ischemic stroke 2018. *Chin J Neurol.* (2018) 51:666–82. doi: 10.3760/cma.j.issn.1006-7876.2018.09.004

35. Fei YT, Cao HJ, Xia RY, Chai QY, Liang CH, Feng YT, et al. Methodological challenges in design and conduct of randomised controlled trials in acupuncture. *BMJ.* (2022) 376:e64345. doi: 10.1136/bmj-2021-064345

36. Zhang GS, Zhang CS, Tan HY, Wang Y, DaCosta C, Zhang AL, et al. Systematic review of acupuncture placebo devices with a focus on the credibility of blinding of healthy participants and/or acupuncturists. *Acupunct Med.* (2018) 36:204–14. doi: 10.1136/acupmed-2017-011484

frontiers | Frontiers in Neurology

TYPE Review
PUBLISHED 28 October 2022
DOI 10.3389/fneur.2022.1030747

OPEN ACCESS

EDITED BY
Lingyong Xiao,
First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China

REVIEWED BY
Zhigang Mei,
Hunan University of Chinese Medicine, China
Man Li,
Huazhong University of Science and Technology, China
Yang Tiansong,
Heilongjiang University of Chinese Medicine, China

*CORRESPONDENCE
Jing-Wen Yang
yangjw0626@126.com

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 29 August 2022
ACCEPTED 10 October 2022
PUBLISHED 28 October 2022

CITATION
Wang L, Su X-T, Cao Y, Yang N-N, Hao X-W, Li H-P, Wang Q-Y and Yang J-W (2022) Potential mechanisms of acupuncture in enhancing cerebral perfusion of ischemic stroke.
Front. Neurol. 13:1030747.
doi: 10.3389/fneur.2022.1030747

Potential mechanisms of acupuncture in enhancing cerebral perfusion of ischemic stroke

Lu Wang, Xin-Tong Su, Yan Cao, Na-Na Yang, Xiao-Wan Hao, Hong-Ping Li, Qing-Yong Wang and Jing-Wen Yang*

School of Acupuncture-Moxibustion and Tuina, International Acupuncture and Moxibustion Innovation Institute, Beijing University of Chinese Medicine, Beijing, China

Ischemic stroke is the predominant cause of long-term disability and death worldwide. It is attributable to the sudden interruption of regional cerebral blood flow, resulting in brain cell death and neurological impairment. Acupuncture is a widely used adjuvant treatment for ischemic stroke in China and shows promising efficacy in clinical practice. This review mainly focused on the evidence to illustrate several possible mechanisms of acupuncture therapy on cerebral perfusion in ischemic stroke. Studies have shown that acupuncture is probably effective in the enhancement of cerebral perfusion after ischemic stroke. It promotes the improvement of hemodynamics, the release of vasoactive substances, the formation of new blood vessels, as well as the restitution of microcirculation. Multiple factors may contribute to the variability in acupuncture's therapeutic effects, including the acupoint selection, stimulation frequency and intensity, and retaining needle time. Acupuncture has the potential to become a non-pharmacological adjuvant approach to enhance cerebral perfusion in ischemic stroke. Future studies are required to gain our insight into acupuncture as well as accelerate its clinical translation.

KEYWORDS

acupuncture, ischemic stroke, cerebral perfusion, hemodynamics, vasoactive substances, angiogenesis, microcirculation

Introduction

Ischemic stroke is a significant cause of morbidity and mortality worldwide and usually occurs when an artery supplying the brain becomes occluded (1). The substantial reduction of blood flow brings about the insufficient delivery of glucose and oxygen to the affected tissues, which ultimately results in the death of brain tissue and focal neurological deficits. Brain damage after ischemic stroke can be limited by rescuing the ischemic penumbra, which is severely hypoperfused and hypoxic, at-risk but not yet infarcted tissue (2). Evidence-based treatments to salvage the penumbra involve restoring blood flow as early as

possible; otherwise, over time, the penumbra evolves into a core of irreversibly damaged tissue until it disappears completely. Therefore, timely revascularization therapies comprising intravenous thrombolysis and mechanical thrombectomy are the effective primary treatments for early ischemic stroke and are beneficial to improve the prognosis of neurological function, which are recommended by the current clinical guidelines (3, 4). However, their use is subject to a number of limitations, including the short time window, different approval restrictions, and many contraindications (2, 5).

Several non-pharmacological interventions, such as decompressive surgery (6), normobaric oxygen therapy (7), hypothermia (8), transcranial laser treatment (9), and sensory stimulation (10), showed great promise to slow down the demise of the penumbra as an adjunct to intravenous thrombolysis and endovascular thrombectomy and to improve functional outcomes. By constraining infarct growth, these interventions might also make patients generally excluded from intravenous alteplase and endovascular therapy eligible for this treatment, thereby increasing the number of treated patients, not only within but also beyond the currently approved time window (11). Acupuncture, as the primary method of treating diseases in Traditional Chinese Medicine for over 3,000 years, was a frequently applied non-pharmacological therapy that was claimed to be effective in treating strokes in many hospitals in China. Experimental studies showed its potential beneficial effects for ischemic stroke rehabilitation *via* benign regulation of oxidative stress (12), glutamate excitotoxicity (13), inflammation (14), apoptosis (15), and autophagy (16). Of note, increasing studies have indicated that acupuncture seems to be a helpful treatment for cerebral blood flow (CBF) enhancement following an ischemic stroke. However, the underlying mechanisms have not been well-understood to date. In this review, we focused on the evidence to illuminate how acupuncture had beneficial effects on CBF and its underlying mechanisms.

Materials and methods

Search strategy

Relevant studies were retrieved from PubMed database from 2000 to the present. Search terms consisted of three groups: interventions ["acupuncture" OR "electroacupuncture"], diseases ["ischemic stroke" OR "cerebral ischemia" OR "cerebral infarction"], and indicators ["cerebral perfusion" OR "cerebral blood flow" OR "collateral circulation" OR "brain circulation" OR "microcirculation" OR "hemodynamics" OR "angiogenesis" OR "blood viscosity" OR "blood flow velocity" OR "vasoactive substances" OR "vascular reactivity"].

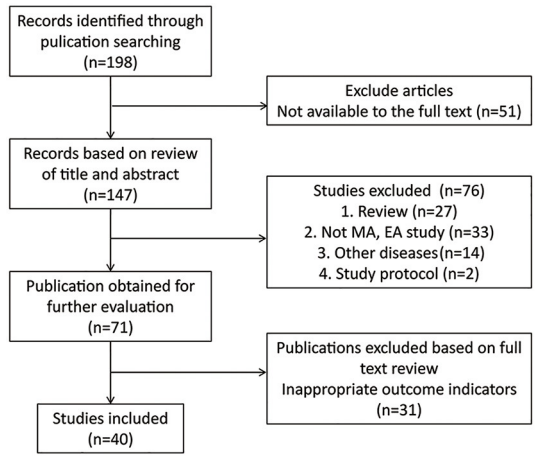


FIGURE 1
Flow chart of study selection. MA, manual acupuncture; EA, electroacupuncture.

Study selection

We found 198 potentially relevant literatures *via* searching on an online database and of these articles, we excluded 158 articles for the following reasons: (1) full text was not available; (2) review; (3) studies that did not use manual acupuncture (MA) and electroacupuncture (EA); (4) other diseases; (5) study protocol; and (6) inappropriate outcome indicators. At last, 40 articles were included in this study. A flow chart of the search and filter process is shown in Figure 1.

Acupuncture mechanisms on cerebral perfusion in ischemic stroke

Acupuncture modulates cerebral hemodynamics

During ischemic stroke, the CBF decreases and the autoregulation of the cerebral vascular system is damaged, leading to cerebral ischemia and hypoxia, and this may lead to a poor prognosis. Accumulating evidence showed that acupuncture had a positive impact on cerebral hemodynamics. A randomized controlled trial showed that acupuncture significantly increased CBF velocity in patients with ischemic stroke, and the increase continued 5 min after needles were removed (17). Acupuncture elevated mean blood flow velocity and maximum peak flow speed, and lowered the vascular resistance index in patients with ischemic stroke (18). In addition, single-photon emission computed tomography was used for comparison brain perfusion images before and after acupuncture in patients with middle cerebral artery occlusion

(MCAO). The results suggested that acupuncture could enhance regional CBF, peculiarly in the low perfusion area around the ischemic core, ipsilateral, or contralateral sensorimotor area (19). Another study reported that acupuncture was able to reduce higher shearing rate and lower shearing rate of whole blood viscosity, plasma viscosity, hematocrit, and profibrin in patients with ischemic stroke (20). Animal experiments showed similar results. After 15 min of MCAO in monkeys, EA for 1 h enhanced the local CBF in the striatum significantly (21). EA was administered for 15 min beginning 2 h after unilateral local ischemic infarction of the primary motor cortex was established in mice. The results revealed that EA rescued stroke-induced impairment of blood perfusion and neuronal activity in the contralateral primary motor cortex and primary sensory cortex (22). Moreover, EA for 20 min accelerated the CBF in the middle cerebral artery area of rats with bilateral common carotid artery occlusion (23). Likewise, acupuncture for 5 s recovered regional CBF in the ischemic cortex of MCAO rats (24) (Table 1). These findings suggest that acupuncture can be effective in enhancing cerebral hemodynamics by regulating blood flow velocity, vascular resistance index, blood viscosity during ischemic stroke.

Acupuncture modulates the release of vasoactive substances

Acetylcholine (ACh), considered a crucial mediator of cerebral vasodilation, can activate endothelial nitric oxide synthase (eNOS) by binding to M-type cholinergic receptors on the surface of the vascular endothelium and produce an appropriate amount of NO on smooth muscle cells to relax blood vessels. The central cholinergic system is susceptible to ischemia, and even mild hypoxia may impair the synthesis of ACh. 60 min post-MCAO, EA for 20 min prominently elevated the perfusion of the cerebral cortex. Cerebral perfusion began to increase at 10 s after EA stimulation, gradually enhanced during the stimulation process, and lasted until 20 min after the end of EA. Nevertheless, the increase of perfusion induced by EA was completely blocked by the M receptor blocker or eNOS gene knockout. It suggests that EA can alleviate the cerebrovascular damage in the acute phase of focal cerebral ischemia, at least in part due to the enhancement of cerebral perfusion in an ACh/eNOS-dependent manner (32). Consistent with this result, it was discovered that EA continuously and steadily built up CBF in the ischemic area, accompanied by an increase in the expression of choline acetyltransferase (ChAT) and five muscarinic receptor subtypes (33).

Angiotensin II (Ang II), as the main bioactive peptide of the brain renin-angiotensin system, plays an essential part in the pathophysiology of ischemic stroke. It has been declared that Ang II combined with Ang II type 1 receptor (AT1R), splits Phosphatidylinositol (4,5)-bisphosphate into diacylglycerol (DAG) and inositol triphosphate (IP3) that leads to the release of Ca^{2+} and mediates vasoconstriction. EA was found to reverse the upregulation of the IP3 signal transduction pathway *via* inhibiting Ang II binding to AT1R, which improved the blood supply of ischemic areas and had a beneficial effect on cerebral ischemia (34). Likewise, the other study found that EA effectively decreased the over-expression of DAG, IP3, and calmodulin in rats with acute cerebral infarction, improved cerebral autonomy movement, and alleviated cerebral vascular spasm (35).

Moreover, endothelin-1 (ET-1) is by far the most powerful vasoconstriction peptide in both arteries and veins, which has a robust contractile effect on vascular smooth muscle. Scalp acupuncture inhibited the increase of peripheral plasma ET-1 in patients with cerebral infarction, contributing to the improvement of vascular elasticity and cerebral blood circulation as well as the reduction of the disability rate (36) (Figure 2 and Table 2).

Therefore, the potential efficacy of acupuncture in ischemic stroke may be its influence on the endothelial nitric oxide system and brain renin-angiotensin system, which can enhance cerebral perfusion by affecting cerebrovascular reactivity.

Acupuncture modulates angiogenesis

The collateral circulation in the initial phase of ischemic stroke relies on the opening of a preexisting vascular network, while in the later period, it mainly depends on the formation of new blood vessels. Angiogenesis takes place within 12 to 24 h following ischemic insults and persists for at least 3 weeks. The central link of angiogenesis is the proliferation, migration, differentiation, and lumen formation of vascular endothelial cells (ECs). Endothelial progenitor cells (EPCs) primarily exist in the bone marrow (BM) and can enter the brain to differentiate into mature ECs, leading to neovascularization (37). EA promoted the increase of the number of EPCs in peripheral blood (PB) and BM at each phase of cerebral ischemia, modulating the mobilization, migration and homing of EPCs (38). It was observed that the ECs of MCAO rats began to proliferate within 24 h, reached the peak on the third day, and decreased on the 7th day. After EA, ECs proliferation accelerated to 12 h, and the number of ECs on the first, second, third, and 7th day was remarkably higher than that in MCAO rats (39). Angiogenesis is a dynamic and complex process engaged in a variety of cytokines and cellular components. In the microenvironment of vascular ECs, angiogenic factors and antiangiogenic factors are always keeping a dynamic balance and jointly regulate the formation of neovascularization.

TABLE 1 Acupuncture modulates cerebral hemodynamics.

Subjects	Acupoint	Method	Frequency intensity	Duration	Treatment course	CBF detection technology	Effects of acupuncture	References
Patients with ischemic stroke	LR3, LR4, SJ5, GB34	MA	-	20 min	Only once	TCD	Vm ↑	(17)
Patients with dysphagia in ischemic stroke	GV11/14/16/20/24/26	MA	Twist 30 s	30 min	5 days a week for 4 weeks	TCD	Vs, Vm ↑, RI ↓	(18)
Patients with MCAO	LI4/10/11/15/16, SJ5	MA	-	20 min	Only once	SPECT	CBF ↑	(19)
Patients with ischemic stroke	LI4/11/15, ST32/36/41, LR3, SJ5	EA	2 HZ, 2–6 mA	20, 40, or 60 min	10 days	rotary viscosity meter, capillary viscometers	whole blood low/high shear viscosity, plasma viscosity, hematocrit, fibrinogen ↓	(20)
MCAO monkeys	GV20/26	EA	−18/3.85 Hz, −7–8.8 mA, 6–8.8 mA	60 min	Only once	LDF	CBF ↑	(21)
Photochemical mice	GV14, GV20	EA	2/10 Hz, 1 mA	15 min	Only once	LSBFI	entire brain, contralateral, and S1 CBF↑	(22)
BCCAO rats	ST36, SP6	EA	2/15 Hz, 5 mA	30 min	Only once	LDF	CBF ↑	(23)
MCAO rats	GV26	MA	3 Hz	5 s	Two times a day for 3 consecutive days	LDF	CBF ↑	(24)
MCAO rats	PC6	EA	3 Hz	5, 60 or 180 s	Six times in 72 h	LDF	CBF ↑	(25)
MCAO rats	PC6	MA	twist 3 times/s	60 s	Five times in 60 h	LDF	CBF ↑	(26)
MCAO rats	GV11/16	EA	7 Hz, 6 mA	30 min	Pre-, intra- or post-ischemia	LDF	CBF ↑	(27)
MCAO rats	GV26, PC6, LU5, SP6, BL40	MA	Twist three times /min	5 s	Six times in 72 h	LDF	CBF ↑	(28)
MCAO rats	GV20/26, LI11, PC6, GB34, SP6	EA	5/20 Hz	5 min	Only once	LDF	CBF ↑	(29)
MCAO rats	GV20/26	EA	5/20 Hz, 1 mA	5, 15, 30, or 45 min	Only once	LDF	CBF ↑	(30)
MCAO rats	PC6	MA	1, 2, 3 Hz	5, 60, 180 s	Only once	LDF	CBF ↑	(31)

CBF, cerebral blood flow; MA, manual acupuncture; Vm, average blood flow velocity; TCD, transcranial Doppler ultrasound; Vs, maximum peak velocity; RI, Resistance index; MCAO, middle cerebral artery occlusion; SPECT, single-photon emission computed tomography; EA, electroacupuncture; LDF, laser Doppler flowmetry; LSBFI, laser speckle blood flow imaging; BCCAO, bilateral common carotid artery occlusion; LR3, Taichong; LR4, Zhongfeng; SJ5, Waiguan; GB34, Yanglingquan; GV11: Shendao; GV14, Dazhui; GV16, Fengfu; GV20, Baihui; GV24, Shenting; GV26, Shuigou; LI14, Binao; LI10, Shousanli; LI11, Quchi; LI15, Jianyu; LI16: Jugu; ST32, Futu; ST36, Zusanli; ST41, Jiexi; SP6, Sanyinjiao; PC6, Neiguan; LU5, Chize; BL40, Weizhong; ↑, upregulation; ↓, downregulation.

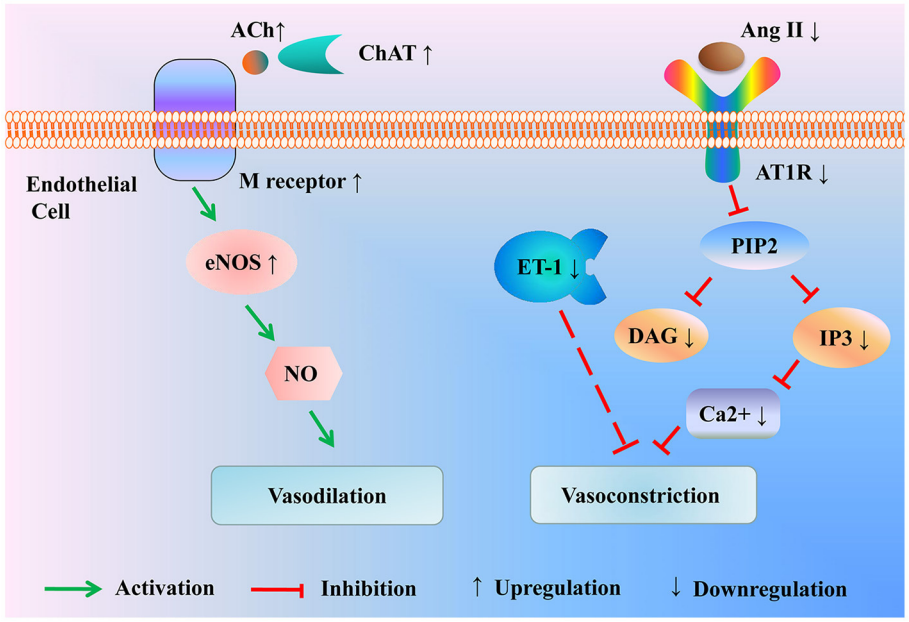


FIGURE 2
Modulation of the vasoactive substance by acupuncture for treating ischemic stroke. Acupuncture enhances the expression of ChAT to promote the activation of the ACh/eNOS signal pathway, resulting in vasodilation. Besides, acupuncture drops the level of Ang II and AT1R to inhibit the activation of the IP3 signal pathway, thus mitigating vasoconstriction. In the meantime, acupuncture alleviates the production of vasoconstrictor factor ET-1. ACh, Acetylcholine; ChAT, choline acetyltransferase; eNOS, endothelial nitric oxide synthase; Ang II, Angiotensin II; AT1R, Ang II type 1 receptor; PIP2, phosphatidylinositol (4,5)-bisphosphate; DAG, diacylglycerol; IP3, inositol triphosphate; ET-1, endothelin-1.

TABLE 2 Acupuncture modulates the release of vasoactive substance.

Subjects	Acupoint	Method	Frequency intensity	Duration	Treatment course	CBF detection technology	Effects of acupuncture	References
MCAO mice	GV14/20	EA	2 Hz, 1 mA	20 min	Only once	LDF	ACh/eNOS↑, CBF↑	(32)
MCAO rats	GV14/20	EA	2/15 Hz, 1 mA	30 min	Only once	LDF	ChAT, five muscarinic receptor subtypes ↑	(33)
MCAO rats	GV26	EA	15 Hz, 1 mA	20 min	Only once	LDF	Ang II, AT1R, DAG, IP3, CaM ↓, CBF ↑	(34)
MCAO rats	GV26	EA	15 Hz, 1 mA	20 min	Only once	LSCM	Gq, DAG, IP3, CaM↓, CBF↑	(35)
Patients with cerebral infarction	LI4/11, ST34/36/41, SJ5, GB20, LR3	MA	twist once every 10 min	30 min	10 days	-	ET-1↓	(36)

CBF, cerebral blood flow; MCAO, middle cerebral artery occlusion; EA, electroacupuncture; LDF, laser Doppler flowmetry; ACh, Acetylcholine; eNOS, endothelial nitric oxide synthase; ChAT, choline acetyltransferase; Ang II, Angiotensin II; AT1R, AngII type 1 receptor; DAG, diacyl glycerol; IP3, inositol triphosphate; CaM, calcium/calmodulin-dependent protein; LSCM, laser scanning confocal microscope; MA, manual acupuncture; ET-1, endothelin-1; GV14, Dazhui; GV20, Baihui; GV26, Shuigou; LI4, Hegu; LI11, Quchi; ST34, Liangqiu; ST36, Zusanli; ST41, Jiexi; SJ5, Waiguan; GB20, Fengchi; LR3, Taichong; ↑, upregulation; ↓, downregulation.

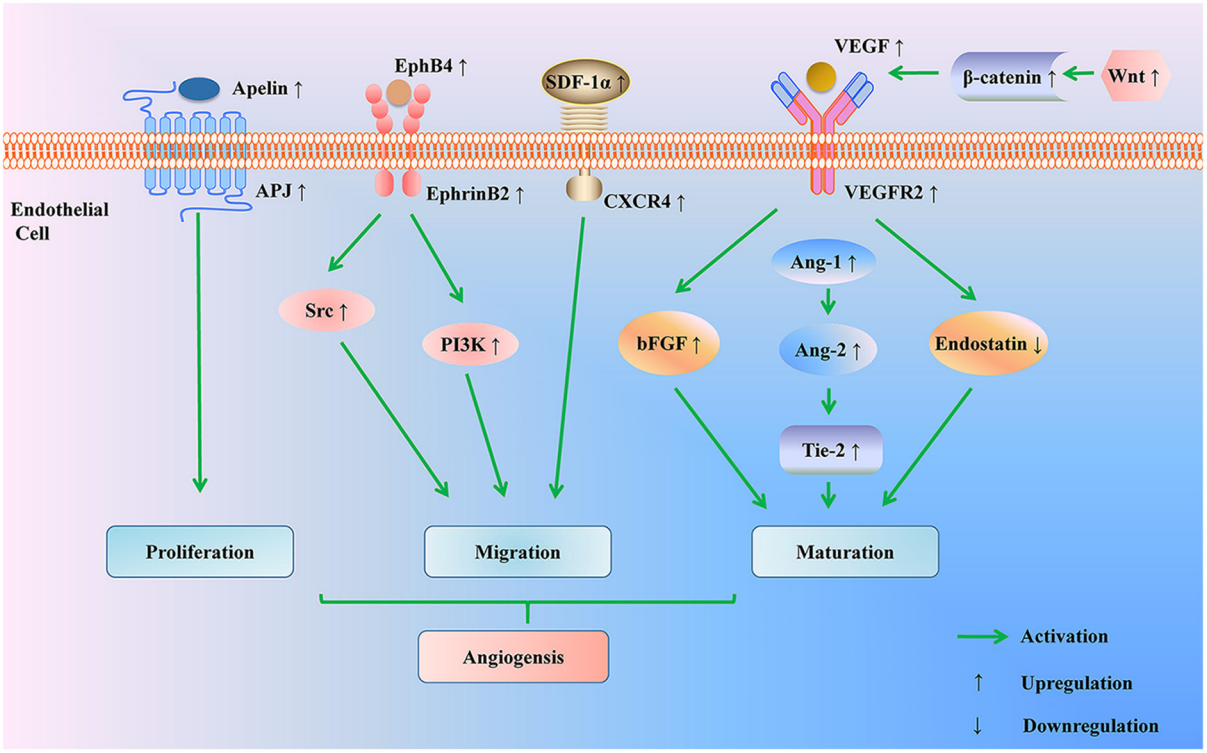


FIGURE 3
Regulation of angiogenesis pathway by acupuncture for treating ischemic stroke. Acupuncture promotes angiogenesis through multiple pathways: acupuncture up-regulates Apelin-APJ system to induce endothelial cells proliferation; acupuncture promotes endothelial cells migration by modulating the EphB4/EphrinB2 signaling pathway and SDF-1/CXCD4 axis; acupuncture activates the Wnt/β-catenin signaling pathway to elevate the expressions of angiogenic factors (VEGF, bFGF, Ang-1, Ang-2, and Tie-2) and to reduce the level of anti-angiogenic factor (endostatin). PI3K, phosphoinositide 3 kinase; SDF-1α, stromal cell-derived factor-1 α; CXCR4, CXC chemokine receptor 4; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor-2; bFGF, basic fibroblast growth factor; Ang-1, Angiopoietin-1; Ang-2, Angiopoietin-2; tyrosine kinase receptor 2 (Tie-2).

Vascular endothelial growth factor (VEGF) pathway

VEGF is deemed to be the most critical factor in modulating angiogenesis. Recent studies have proved that acupuncture elevated the level of VEGF in plasma and cerebral ischemic tissue, which increased EPCs number and improved endothelial function in MCAO rats or patients with ischemic stroke (40–42). Moreover, the cerebral cortical miRNA profile in MCAO rats identified that the VEGF signaling pathway was most prominently affected by EA (43). The angiopoietin/tyrosine kinase receptor 2 (Ang/Tie-2) system was considered a well-suited complement for VEGF: VEGF induced vascular budding while Ang/Tie-2 system promoted maturation of vessels (44). Endostatin is a vascular inhibitor, antagonizing the effect of VEGF (45). It was reported that EA up-regulated the expression of VEGF, Ang-1, Ang-2, and Tie-2 in the cortex round the ischemic necrotic region. Meanwhile, EA decreased the level of endostatin protein in the ischemic penumbra of focal cerebral ischemic rats (46, 47). Moreover, VEGF binds specifically to VEGF receptor-2 (VEGFR-2) on the surface of vascular ECs, activating intracellular signal transduction pathways and promoting neovascularization (48). Basic fibroblast growth factor (bFGF) encourages angiogenesis synergistically with VEGF. It was reported that EA enhanced the levels of VEGF, VEGFR2, and bFGF, thus inducing the growth of microvessels and the increase of regional CBF in the ischemic brain tissue (49). Another studies indicated that the classical Wnt/β-catenin signal pathway played crucial roles in maintaining BBB homeostasis and promoting vascularization by synergistic effects with angiogenesis-related factors. EA could activate the Wnt/β-catenin signaling pathway, thereby elevating the expressions of angiogenic factors including VEGF, bFGF, and Ang-2 and restoring blood perfusion in the ischemic zone (50). Besides, compared with the rats that received only thrombolysis 6 h after the embolic stroke model was established, the rats that received acupuncture for 30 min 2 h after stroke and before thrombolysis showed lower neurologic scores, less infarct volumes, and more expressions of VEGF and bFGF in ischemic areas of the cerebral cortex, indicating that acupuncture was able to prolong the time window of

thrombolysis in cerebral infarction rats, which may be connected with the up-regulation of VEGF and bFGF expressions in ischemic cerebral cortex (51).

EphB4/EphrinB2 pathway

EphB4/EphrinB2 was reported to regulate the migration of ECs and angiogenesis by activation of Src and phosphoinositide 3 kinase (PI3K) signaling pathways. EA could accelerate capillary formation in the brain infarction area of MCAO rats through up-regulating EphB4 and EphrinB2 mRNA and increasing the level of Src and PI3K, manifesting that the formation of new vessels after EA was partly to regulated by EphB4/EphrinB2 mediated Src/PI3K signal pathway (52).

Stromal cell-derived factor-1 α (SDF-1α)/ CXC chemokine receptor 4 (CXCR4) pathway

SDF-1α is also considered an essential factor in angiogenesis by inducing the migration of EPCs. Following cerebral ischemia, the level of SDF-1α was reduced in BM and increased in PB. EA accelerated the formation of SDF-1α concentration gradient and increased the number of new blood vessels (38). Further study demonstrated that SDF-1α was bound to CXCR4 on the surface of EPCs (53). EA promoted cerebral angiogenesis *via* regulating SDF-1α/CXCR4 axis (54).

Apelin/APJ pathway

Apelin-APJ system is involved in the proliferation of vascular smooth muscle cells and inhibits the high vascular permeability caused by VEGF (55). EA increased the expression of Apelin, APJ mRNA, and protein in the cerebral vascular endothelium of MCAO rats in 1, 9, 12, 24 h and 3, 7, 12 days, facilitating angiogenesis after cerebral ischemia (56) (Figure 3 and Table 3).

The evidences above show that acupuncture can trigger angiogenesis through a variety of signaling pathways, thus having a positive impact on cerebral perfusion.

Acupuncture modulates microcirculation

The primary structural and functional basis of the cerebral microcirculation is the blood-brain barrier, composed of microvasculature ECs sealed together by tight junctions, basement membranes, and astrocytes. After cerebral ischemia, the microvascular are usually narrowed or blocked by compression ascribed to the swelling of the foot process of astrocytes. Moreover, under the action of plasminogen activator inhibitor-1 secreted by vascular ECs, red blood cells, white blood cells, platelets, and fibrin are deposited in the narrow blood vessels, thus forming a microthrombus and blocking the microvessels. The microcirculation disturbance is a dominating cause of low perfusion and no reflow. As a consequence, the improvement of microcirculation is of great significance in the metabolism of tissue cells and the recovery of neurological function after ischemia.

When the brain is hypoxic-ischemic, glutamate produced by neurons combines with metabotropic glutamate receptors in astrocytes, inducing the expression of arachidonic acid cytochrome P450 epicytoxygenase (CYP2C11) through signal transduction. CYP2C11 catalyzes arachidonic acid to generate epicosane trienoic acids (EETs), that act on vascular smooth muscle, contributing to dilating microcirculatory vessels and increasing blood perfusion (59). CYP2C11 is a crucial enzyme in amino acid metabolism that indirectly reflects the ability of astrocytes to release EETs. EA raised blood flow in the pial meningeal microcirculation of MCAO rats by up-regulating the expression of CYP2C11 mRNA (60). Furthermore, acupuncture effectively dilated the diameter of microvessels and augmented the blood flow of the leptomeningeal microcirculation (61, 62) (Table 4). Therefore, it is reasonable to infer that acupuncture may enhance microcirculation through the regulation of key enzymes in cell metabolism, thereby improving neurological function prognosis in ischemic stroke.

Factors associated with the effect of acupuncture on cerebral perfusion

Acupoint selection

Among the 40 articles included in this review, the three most frequently selected acupoints to include: (1) Shuigou (GV26), (2) Baihui (GV20), and (3) Neiguan (PC6) (Figures 4, 5). A study compared the difference of CBF regulation effect of Shuigou (GV26), Baihui (GV20), Neiguan (PC6), Quchi (LI11), Yanglingquan (GB34), and Sanyinjiao (SP6) acupoints on MCAO rats. The result showed that EA at Shuigou (GV26) and Baihui (GV20) exerted the best effects, which caused a striking increase in CBF, and the enhancement of blood perfusion was synchronized with EA (29). In another study, acupuncture was applied to Shuigou (GV26), Neiguan (PC6), Sanyinjiao (SP6), Chize (LU5), and Weizhong (BL40) acupoints in MCAO rats; the result identified that only Shuigou (GV26) and Neiguan (PC6) had a significant effect on restoring CBF (28). Besides, it was discovered that the pericardial meridian acupoint group [Quze (PC3) and Neiguan (PC6)] was notably superior to that of the large intestinal meridian acupoint group (Hegu (LI4) and Quchi (LI11)) in facilitating microangiogenesis (41).

Stimulation frequency and intensity

Different stimulation frequency and intensity produced different effects on cerebral perfusion after ischemic stroke. EA at Shuigou (GV26) and Baihui (GV20) was given in diverse

TABLE 3 Acupuncture modulates angiogenesis.

Subjects	Acupoint	Method	Frequency intensity	Duration	Treatment course	CBF detection technology	Effects of acupuncture	References
Patients with cerebral infarction	ST36/37, PC5, and PC6 on the left side	EA	2 Hz	20 min	Only once	-	ECs number ↑, VEGF ↑	(40)
MCAO rats	LI4, PC3, PC6, LI11	EA	20 Hz, 2–4 V	30 min	6, 24, 48, and 72 h after MCAO	-	VEGF ↑, microvascular number ↑	(41)
MCAO rats	GV20, GV26, ST36	EA	2/15 Hz, 1 mA	30 min	Once daily for 1, 2, 4, or 8 days	-	VEGF mRNA↑	(42)
MCAO rats	LI4	EA	40/60 Hz, 1.5 V	15 min	7 days	-	VEGF, Ang-1 ↑, Endostatin ↓	(46)
MCAO rats	GV26	EA	15 Hz, 0.1 mA	20 min	3, 6, 12, 24 h 3, 7, 12 d	-	Ang-1, Ang-2, Tie-2 ↑	(47)
MCAO rats	GV20, GV26, PC6	EA	2/20 Hz, 3–5 V	20 min	14 days	LDF	VEGF, VEGFR2, bFGF ↑	(49)
MCAO rats	GV20, GV26, PC6	EA	2/20 Hz, 3–5 V	20 min	14 days	-	VEGF, bFGF ↑	(57)
MCAO rats	GV26	EA	15 Hz, 0.1 mA	20 min	one time per day in 2, 3, 7, and 12 day	LDF	ECs number ↑, CBF in the ischemic boundary region ↑	(39)
MCAO rats	GV26, PC6	EA	2 Hz, 3 mA	1 min	1, 2, or 3 weeks	LDF	alter miRNA, CBF ↑	(43)
MCAO rats	GV26	EA	15 Hz, 1 mA	5 min	Only once	LDF	CBF on the infarct and non-infarct sides ↑, number of blood vessels ↑	(58)
MCAO rats	GV20	EA	3–15 Hz 2–4 mA	30 min	21 days	LDF	VEGF, FLK1, bFGF, Ang2↑, Wnt3a, β-catenin, cyclin D1↑	(50)
Embolic stroke rats	GV26, PC6	MA	Twist 1 min	30 min	Only once	-	VEGF, bFGF↑ endostatin↓	(51)
MCAO rats	GV4, GV9, GV14, GV20, GV26	EA	15 Hz	30 min	7 days	-	EphB4, EphrinB, Src, PI3K↑	(52)
MCAO rats	GV20, LI4, LR3	EA	2/20 Hz, 1 mA	30 min	7 days	-	SDF-1α ↑	(38)

(Continued)

TABLE 3 (Continued)

Subjects	Acupoint	Method	Frequency intensity	Duration	Treatment course	CBF detection technology	Effects of acupuncture	References
MCAO rats	GV20, ST36	EA	40 Hz, 1–2 mA	20 min	14 days	-	SDF-1α ↑	(54)
MCAO rats	GV26	EA	15 Hz, 2 mA	20 min	1, 3, 6, 9, 12, or 24 h after MCAO; Once daily for 3, 7, or 12 days after MCAO	-	Apelin-APJ ↑	(56)

CBF, cerebral blood flow; EA, electroacupuncture; ECs, endothelial cells; VEGF, vascular endothelial growth factor; MCAO, middle cerebral artery occlusion; Ang-1, Angiopoietin-1; LDF, laser Doppler flowmetry; VEGFR-2, VEGF receptor-2; bFGF, basic fibroblast growth factor; PI3K, Phosphoinositide 3 kinase; SDF-1α, stromal cell-derived factor-1 α; ST36, Zusanli; ST37, Shangjuxu; PC5, Jianshi; PC6, Neiguan; LI4, Hegu; PC3, Quze; LI11, Quchi; GV20, Baihui; GV26, Shuigou; GV4, Mingmen; GV9, Zhiyang; GV14, Dazhui; LR3, Taichong; ↑, upregulation; ↓, downregulation.

TABLE 4 Acupuncture modulates microcirculation.

Subjects	Acupoint	Method	Frequency intensity	Duration	Treatment course	CBF detection technology	Effects of acupuncture	References
MCAO rats	PC6, LI11	EA	2/15 Hz, 1 mA	20 min	7 days	LDF	CYP2C 11 mRNA ↑ CBF ↑	(60)
MCAO rats	GV15/16/17, GB20	MA	Twist 1 min	15 min	14 days	LDF	Microcirculation ↑, Blood viscosity ↓	(62)
MCAO rats	PC6	MA	Twist 60, 120, 180 times /min	5, 60, or 180's	Only once	LDF, Microcirculation detector	CBF↑	(61)

CBF, cerebral blood flow; MCAO, middle cerebral artery occlusion; EA, electroacupuncture; CYP2C11, arachidonic acid cytochrome P450 epicytoxygenase; MA, manual acupuncture; LDF, laser Doppler flowmetry; PC6, Neiguan; LI11, Quchi; GV15, Yamen; GV16, Fengfu; GV17, Naohu; GB20, Fengchi; ↑, upregulation; ↓, downregulation.

intensities (0–1.2 mA) and frequencies (1–100 Hz). The results were that the frequency of 5-20 Hz produced the best effect, which drastically conduced to the increase of blood perfusion and blood cell concentration (63). When rats were treated with EA at Shuigou (GV26) and Baihui (GV20) with the fixed frequency of 5/20 Hz, 0.6 mA stimulation did not spark off CBF alter, but the blood flow was considerably upregulated from 0.6 mA to 0.8 mA. When it changed to 1.0 mA, the CBF further increased twice as much as before EA, whereas the stimulation intensity of 1.2 mA maintained the same level. Consequently, the optimal intensity of EA stimulation at Shuigou (GV26) and Baihui (GV20) may range from 1.0 and 1.2 mA (63). The combination of varying acupuncture intensity and frequency may produce unlike therapeutic effects. The effects of diverse parameter combinations (0.4 mA, 5/20 Hz), (1.0 mA, 5/20 Hz), (1.0 mA, 70 Hz), on CBF were compared in MCAO rats receiving EA at Shuigou (GV26) and Baihui (GV20). The outcomes showed that the therapeutic effect of 1.0 mA, 5/20 Hz was strikingly better than that of the other two groups, resulting in a manifest increase in CBF (63).

Twisting technique was used in needling at Neiguan (PC6) with different frequencies (60, 120, and 180 times/minute). The neurological deficit score, leptomeningeal blood flow, microcirculation, and cerebral infarction rate were taken as effective indexes. The consequences revealed that the acupuncture parameter of the best acupuncture effect was 180 times/minute, in other words, fast frequency, which had apparent advantages in enhancing CBF and alleviating the rate of cerebral infarction (61).

Retaining needle time

The optimal duration of acupuncture stimulation was of great importance to cerebral perfusion, infarction volume, neurological deficits degree, and mortality. In one study, EA

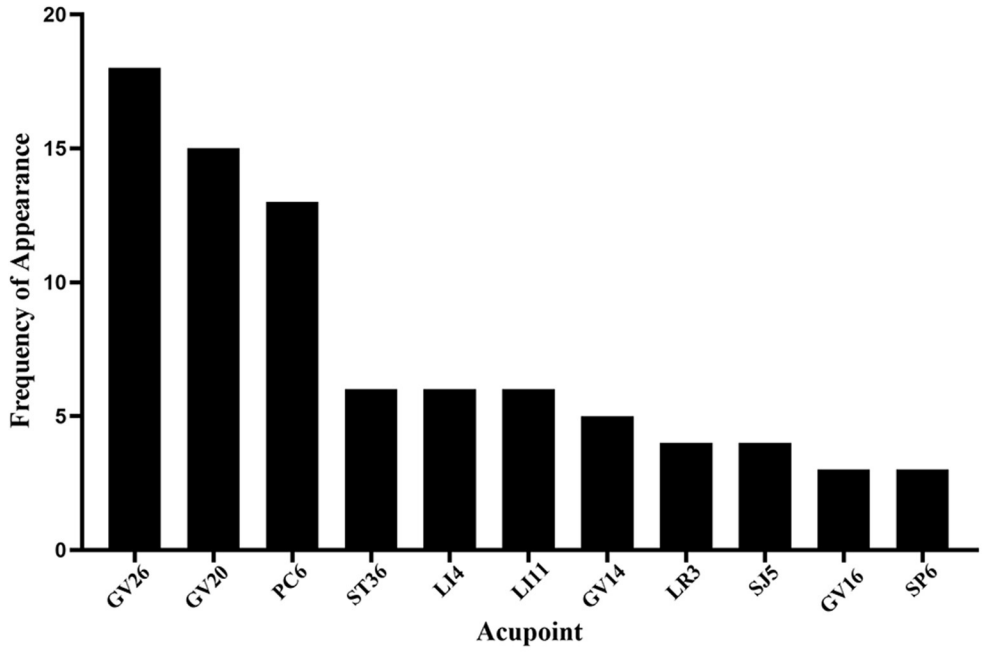


FIGURE 4
Frequency summary of individual acupoint. Only the acupoint that appears more than twice is shown. GV26: Shuigou; GV20: Baihui; PC6: Neiguan; ST36, Zusanli; LI4: Hegu; LI11: Quchi; GV14: Dazhui; LR3: Taichong; SJ5: Waiguan; GV16: Fengfu; SP6: Sanyinjiao.

was delivered to Shuigou (GV26) and Baihui (GV20) with sparse-dense wave (5/20 Hz) at 1.0 mA for 5, 15, 30, and 45 min, respectively. The consequences showed that 30 min of EA noticeably enhanced CBF, reduced the volume of cerebral infarction, and ameliorated the defect of neurological function. Although EA 45 min also elevated the CBF during MCAO, it brought about a worsening of mortality (30). Similar to this result, in the other study, acupuncture was applied to Neiguan (PC6) at a fixed frequency of 3 Hz (180 times/minute) with different durations, i.e., 5, 60 and 180 s. The results demonstrated that the therapeutic effect of acupuncture for 60 s was significantly better than that for 5 s and 180 s, with faster CBF, better recovery of neurological function, and smaller cerebral infarction volume (25).

So far, Shuigou (GV26), Baihui (GV20), and Neiguan (PC6) were the commonly selected acupoints and exerted the better effects on enhancing cerebral perfusion in ischemic stroke. EA at Shuigou (GV26) and Baihui (GV20) with appropriate intensity (1.0 mA), frequency (5/20 Hz), and retaining needle time (30 min) effectively increases the blood flow to the ischemic brain region. Neiguan (PC6) with appropriate twisting-rotating frequency (180 times/minute) and retaining needle time (60 s) showed the highest increase in CBF and the best protective effect on neurological function.

Discussion

Optimizing cerebral perfusion is the key to rescuing salvageable ischemic brain tissue. According to all evidence from the studies we have reviewed, acupuncture showed a beneficial effect on cerebral perfusion in ischemic stroke. During the initial stage of ischemic stroke, acupuncture facilitated the recovery of the CBF through modulating hemodynamic disorders and the release of vasoactive substances. During chronic ischemia, acupuncture promoted the formation of new blood vessels *via* modulating the VEGF, EphB4/EphrinB2, SDF-1α/CXCR4, and Apelin/APJ pathways. In the meantime, acupuncture improved microcirculation, enhancing energy metabolism of brain tissues and ameliorating neurological function prognosis (Figure 6).

There is substantial evidence that CBF is regulated directly by neurovascular nerves. Recently, nerve stimulation therapies, such as facial nerve stimulation (64), trigeminal nerve stimulation (65), and sphenoid palatal ganglion stimulation (66), have been reported to have beneficial effects on decreasing cerebrovascular resistance and upregulating cerebral perfusion in ischemic brain tissue. Acupuncture stimulation is a procedure involving the insertion of a fine needle into the skin or deeper tissues at specific acupoints of the body. Abundant neuroreceptors in the nerve terminals of acupoints are considered the basis of the needling sensation of patients, such as free nerve terminal muscle spindle, annular corpuscles,

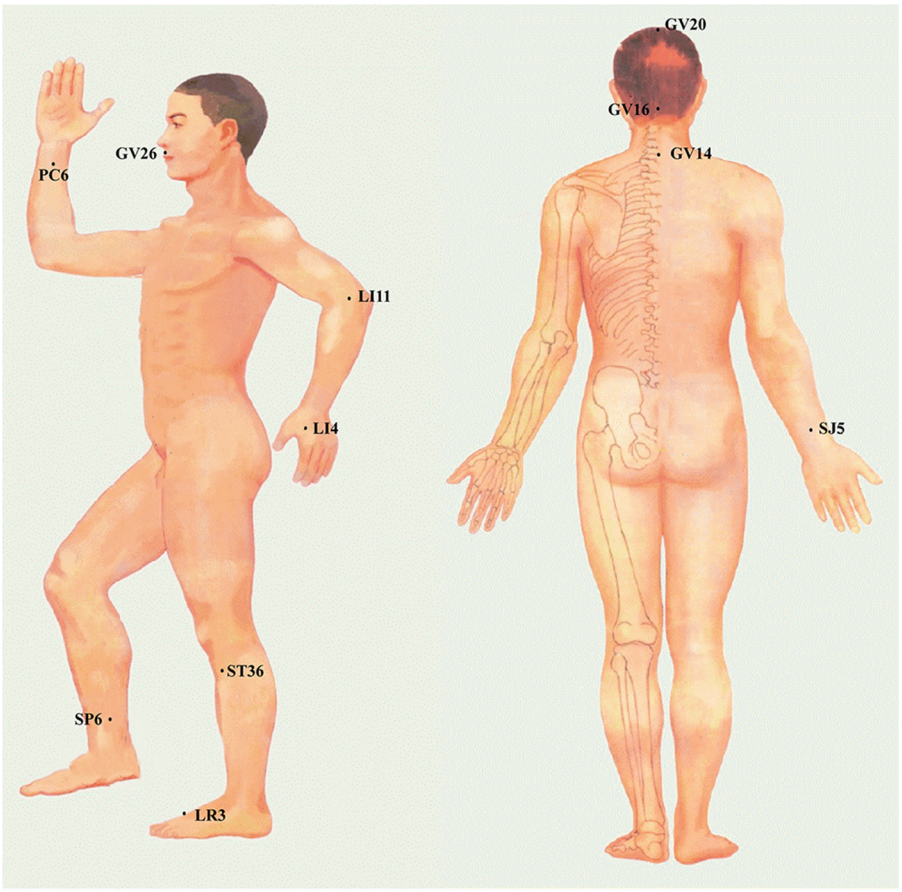


FIGURE 5
The specific location of acupoints is frequently applied to regulate CBF in the ischemic stroke.

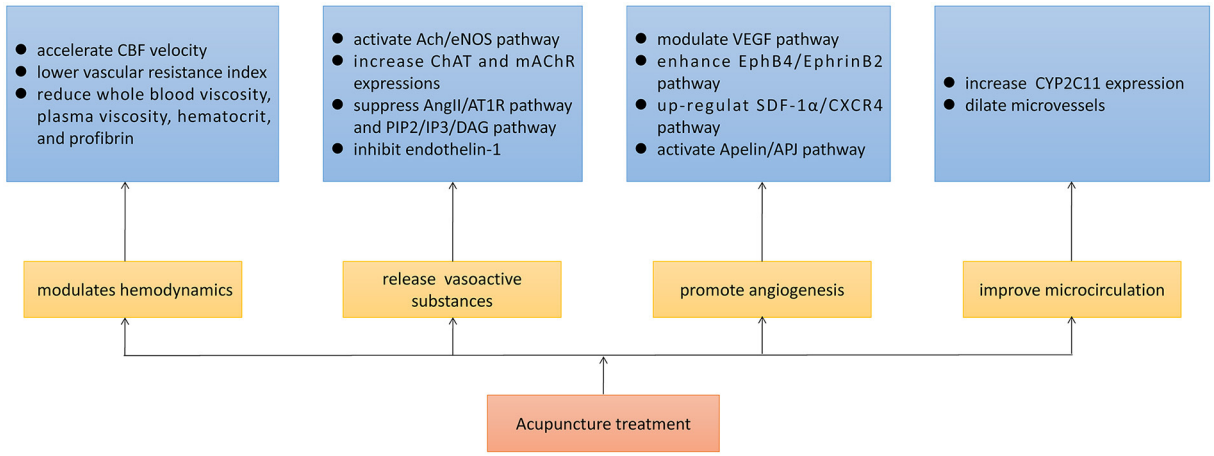


FIGURE 6
Potential mechanisms of acupuncture in enhancing cerebral perfusion of ischemic stroke.

Kirschner's terminal ball, among others (67). After activating receptors located on the neural terminals, acupuncture signals are partly transmitted to the central nervous system, leading to modulation of brain functions (67). The most frequently used Shuigou (GV26) and Baihui (GV20) are distributed in the face and head, respectively located in the afferent sensory nerve fibers innervation range of maxillary branch and ophthalmic branch of the trigeminal nerve. Neiguan (PC6) is situated on the volar side of the wrist and belongs to the range of afferent sensory nerve fibers innervated by the median nerve. Previous studies pointed out that trigeminal nerve stimulation could cause cerebrovasodilation and enhance cerebral perfusion through the trigemino-cerebrovascular system and trigemino-parasympathetic reflex (65). Median nerve stimulation increased the regional CBF of the contralateral motor and somatosensory cortex (68). Notably, one study reported that EA to the ophthalmic branch of the trigeminal nerve enhanced CBF in the prefrontal cortex of healthy subjects (69). Further animal experiment observed that EA stimulation of acupoints on the head and face augmented the CBF in MCAO rats, while this effect disappeared when parasympathetic nerve function was blocked by unilateral vagotomy and atropine (33), suggesting that acupuncture is probably to be a potential nerve stimulation therapy to regulate CBF. However, the specific mechanism regarding how the stimulation signal of acupuncture is delivered from peripheral acupoints to the central nervous system to enhance cerebral perfusion is still unclear. Besides, whether neurovascular coupling is implicated in the regulation of CBF by acupuncture needs to be further explored.

Acupuncture is currently mainly applied clinically during the stroke rehabilitation period, adopted by the National Institutes of Health. Notably, the numerous studies that we have reviewed in this study clarified the beneficial effects of acupuncture therapy on CBF in the acute stage of ischemic stroke. As we all know, the ischemic lesion will evolve during the transport of patients to the endovascular center, and many acute stroke patients do not receive revascularization therapy like thrombolysis or thrombectomy due to the limited time window (2). Acupuncture therapy, with the advantages of non-invasive and easy operation, is available to be delivered at the prehospital stage, in the ambulance, or mobile stroke units, may have the potential to freeze the penumbra and prevent infarct growth, which will probably make the number of patients who can be treated successfully with endovascular therapy or intravenous alteplase substantially increase because the treatment time window may be prolonged. Additionally, not all patients have an opportunity to achieve early initiation of revascularization therapy on account of rigorous eligibility criteria and numerous contraindications (70), even though successful reperfusion after getting these therapeutic modalities is very likely to suffer from the no-reflow phenomenon ascribed to microcirculatory clogging (71). More importantly, the restoration of blood flow in patients received revascularization therapy may result in secondary reperfusion injury (72), a process that involves the production of reactive oxygen species, inflammation, cell apoptosis, and autophagy. Increasing evidence suggests that acupuncture can prevent the generation of excessive reactive oxygen species (73), alleviate the inflammatory response (74), and inhibit apoptosis and autophagy (75) after cerebral ischemia/reperfusion and promote repair of the injured nervous system. Therefore, acupuncture may be a promising auxiliary strategy for revascularization therapy, thereby producing cumulative effects in a synergistic form of treatment in the early phase of acute ischemic stroke.

Even though many advances have been made in further studies, there are still several questions that remain to be elucidated. Firstly, the therapeutic effects of acupuncture are influenced by plenty of variables, such as acupoint selection, stimulation frequency and intensity, and retaining needle time. Although acupuncture therapy emphasizes individuality, lacking standardized treatment regimens will hinder its promotion in clinical practice. Furthermore, there is an obvious disconnect between basic research and clinical research in the selection of acupoints and stimulation parameters, which makes the current research results unable to effectively guide clinical practice. Secondly, most studies reviewed were conducted using animal models, especially MCAO model. However, transient mechanical vascular occlusion is not a model of naturally occurring stroke and its clinical relevance in particular with respect to translational aspects is poor (76). Choosing the most appropriate stroke model might increase the extrapolation of animal data to humans. Thirdly, numerous studies apply laser Doppler flowmetry or transcranial Doppler ultrasound to monitor CBF. While these approaches are economical and convenient, they have low resolution and are prone to false positives. More advanced techniques should be applied, such as single-photon emission computed tomography, arterial spin labeling, perfusion-weighted imaging, digital subtraction angiography, CT angiography, which can more intuitively reflect the influence of acupuncture on cerebral perfusion and cerebral vascular state. Fourthly, to date, most clinical studies focus on the ameliorating effect of acupuncture on neurological dysfunction in ischemic stroke, and only a few trials pay attention to the regulating effect of acupuncture on cerebral blood flow. Besides, convincing evidence from theses clinical trials in support of acupuncture enhancing cerebral perfusion in ischemic stroke were not regarded as robust due to the methodological weaknesses, such as the use of outcome measures that were not internationally recognized, unclear methods of randomization and allocation concealment, the lack of long-term follow-up, and publication bias. Researchers need to consider carrying out more high-quality clinical trials to determine the efficacy of acupuncture used as an adjunct to standard care in ischemic stroke. What's more, fundamental issues concerning the therapeutic window,

duration and mechanisms of action, and the risk of adverse effects also remain to be answered.

In summary, the above-reviewed evidence suggests that acupuncture has a positive impact on cerebral perfusion after ischemic stroke. Renewed efforts are needed to improve our understanding of acupuncture in regulating CBF and to translate these experimental findings to clinical practice.

Author contributions

LW wrote the paper and made the pictures. LW, X-TS, YC, and N-NY searched the literature. LW, X-WH, and YC sorted out the table. J-WY, H-PL, and Q-YW revised the paper. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the National Natural Science Foundation of China (Grant Numbers 82004479 and 81674055).

Acknowledgments

We would like to thank Marc Fisher (Department of Neurology, Beth Israel Deaconess Medical Center, and Harvard Medical School) for giving some positive suggestions for the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics-2016 update: a report from the American heart association. *Circulation.* (2016) 133:e38–360. doi: 10.1161/CIR.0000000000000350

2. Baron JC. Protecting the ischaemic penumbra as an adjunct to thrombectomy for acute stroke. *Nature reviews Neurology.* (2018) 14:325–37. doi: 10.1038/s41582-018-0002-2

3. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American heart association/American stroke association. *Stroke.* (2019) 50:e344–418. doi: 10.1161/STR.0000000000000211

4. Turc G, Bhogal P, Fischer U, Khatri P, Lobotesis K, Mazighi M, et al. European stroke organization (Eso)- European society for minimally invasive neurological therapy (Esmint) guidelines on mechanical thrombectomy in acute ischemic stroke. *J Neurointerv Surg.* (2019) 11:535–8. doi: 10.1136/neurintsurg-2018-014568

5. Mokin M, Kass-Hout T, Kass-Hout O, Dumont TM, Kan P, Snyder KV, et al. Intravenous thrombolysis and endovascular therapy for acute ischemic stroke with internal carotid artery occlusion: a systematic review of clinical outcomes. *Stroke.* (2012) 43:2362–8. doi: 10.1161/STROKEAHA.112.655621

6. Slotty PJ, Kamp MA, Beez T, Beenen H, Steiger HJ, Turowski B, et al. The influence of decompressive craniectomy for major stroke on early cerebral perfusion. *J Neurosurg.* (2015) 123:59–64. doi: 10.3171/2014.12.JNS141250

7. Ejaz S, Emmrich JV, Sitnikov SL, Hong YT, Sawiak SJ, Fryer TD, et al. Normobaric hyperoxia markedly reduces brain damage and sensorimotor deficits following brief focal ischaemia. *Brain J Neurol.* (2016) 139(Pt 3):751-64. doi: 10.1093/brain/awv391

8. Wu TC, Grotta JC. Hypothermia for acute ischaemic stroke. *Lancet Neurol.* (2013) 12:275–84. doi: 10.1016/S1474-4422(13)70013-9

9. Hacke W, Schellinger PD, Albers GW, Bornstein NM, Dahlof BL, Fulton R, et al. Transcranial laser therapy in acute stroke treatment: results of neurothera effectiveness and safety trial 3, a Phase Iii clinical end point device trial. *Stroke.* (2014) 45:3187–93. doi: 10.1161/STROKEAHA.114.005795

10. von Bornstädt D, Gertz K, Lagumersindez Denis N, Seners P, Baron JC, Endres M. Sensory stimulation in acute stroke therapy. *J Cereb Blood Flow Metab.* (2018) 38:1682–9. doi: 10.1177/0271678X18791073

11. Albers GW, Lansberg MG, Kemp S, Tsai JP, Lavori P, Christensen S, et al. A multicenter randomized controlled trial of endovascular therapy following imaging evaluation for ischemic stroke (Defuse 3). *Int J Stroke J Int Stroke Soc.* (2017) 12:896–905. doi: 10.1177/1747493017701147

12. Su XT, Wang L, Ma SM, Cao Y, Yang NN, Lin LL, et al. Mechanisms of acupuncture in the regulation of oxidative stress in treating ischemic stroke. *Oxid Med Cell Longev.* (2020) 2020:7875396. doi: 10.1155/2020/7875396

13. Yang C, Liu J, Wang J, Yin A, Jiang Z, Ye S, et al. Activation of astroglial Cb1r Mediates cerebral ischemic tolerance induced by electroacupuncture. *J Cereb Blood Flow Metab.* (2021) 41:2295–310. doi: 10.1177/0271678X21994395

14. Cao BQ, Tan F, Zhan J, Lai PH. Mechanism underlying treatment of ischemic stroke using acupuncture: transmission and regulation. *Neural Regen Res.* (2021) 16:944–54. doi: 10.4103/1673-5374.297061

15. Chen B, Lin WQ Li ZF, Zhong XY, Wang J, You XF, et al. Electroacupuncture attenuates ischemic brain injury and cellular apoptosis *via* mitochondrial translocation of cofilin. *Chin J Integr Med.* (2021) 27:705–12. doi: 10.1007/s11655-021-3335-4

16. Chen C, Yu Q, Xu K, Cai L, Felicia BM, Wang L, et al. Electroacupuncture pretreatment prevents ischemic stroke and inhibits wnt signaling-mediated autophagy through the regulation of Gsk-3β phosphorylation. *Brain Res Bull.* (2020) 158:90–8. doi: 10.1016/j.brainresbull.2020.03.002

17. Ratmansky M, Levy A, Messinger A, Birg A, Front L, Treger I. The effects of acupuncture on cerebral blood flow in post-stroke patients: a randomized controlled trial. *J Altern Complement Med.* (2016) 22:33–7. doi: 10.1089/acm.2015.0066

18. Xing BF, Zhou X, Deng XQ. Effect of "Tongdu Tiaoshen" needling combined with swallowing training on dysphagia, cerebral blood flow and serum bdnf and ngf levels in ischemic stroke patients. *Zhen Ci Yan Jiu.* (2019) 44:506–11. doi: 10.13702/j.1000-0607.180631

19. Lee JD, Chon JS, Jeong HK, Kim HJ, Yun M, Kim DY, et al. The cerebrovascular response to traditional acupuncture after stroke. *Neuroradiology.* (2003) 45:780–4. doi: 10.1007/s00234-003-1080-3

20. He YZ, Han B, Zheng SF, Wang LN, Chen ZM, Hu J, et al. Effect of different acupuncture needle-retaining time on hemorheology in ischemic stroke patients. *Zhen Ci Yan Jiu.* (2007) 32:338–41.

21. Gao H, Guo J, Zhao P, Cheng J. The neuroprotective effects of electroacupuncture on focal cerebral ischemia in monkey. *Acupunct Electrother Res.* (2002) 27:45–57. doi: 10.3727/036012902816026112

22. Yao LL, Yuan S, Wu ZN, Luo JY, Tang XR, Tang CZ, et al. Contralateral S1 function is involved in electroacupuncture treatment-mediated recovery after focal unilateral M1 infarction. *Neural Regen Res.* (2022) 17:1310–7. doi: 10.4103/1673-5374.327355

23. Hsieh CL, Chang QY, Lin IH, Lin JG, Liu CH, Tang NY, et al. The study of electroacupuncture on cerebral blood flow in rats with and without cerebral ischemia. *Am J Chin Med.* (2006) 34:351–61. doi: 10.1142/S0192415X06003886

24. Li XL, Fan NX, Meng HZ, Shi MX, Luo D, Zhang NY. Specific neuroprotective effects of manual stimulation of real acupoints versus non-acupoints in rats after middle cerebral artery occlusion. *Af J Trad Complement Alternat Med AJTCAM.* (2013) 10:186–95. doi: 10.4314/ajtcam.v10i4.30

25. Zhang C, Wen Y, Fan XN, Tian G, Zhou XY, Deng SZ, et al. Therapeutic effects of different durations of acupuncture on rats with middle cerebral artery occlusion. *Neural Regen Res.* (2015) 10:159–64. doi: 10.4103/1673-5374.150727

26. Zhang C, Wen Y, Fan X, Yang S, Tian G, Zhou X, et al. A microarray study of middle cerebral occlusion rat brain with acupuncture intervention. *Evid Based Complemen Alternat Med eCAM.* (2015) 2015:496932. doi: 10.1155/2015/496932

27. Pang J, Itano T, Sumitani K, Negi T, Miyamoto O. Electroacupuncture attenuates both glutamate release and hyperemia after transient ischemia in gerbils. *Am J Chin Med.* (2003) 31:295–303. doi: 10.1142/S0192415X03000977

28. Qian YF, Fan XN Li YJ, Zhang YN, Wei YY, Zhang X, et al. Effects of acupuncture at different acupoints on cerebral blood flow in cerebral ischemia model rats. *Zhongguo Zhen Jiu.* (2009) 29:213–6.

29. Zhou F, Guo J, Cheng J, Wu G, Sun J, Xia Y. Electroacupuncture and brain protection against cerebral ischemia: specific effects of acupoints. *Evid Based Complement Alternat Med eCAM.* (2013) 2013:804397. doi: 10.1155/2013/804397

30. Zhou F, Guo J, Cheng J, Wu G, Xia Y. Effect of electroacupuncture on rat ischemic brain injury: importance of stimulation duration. *Evid Based Complement Alternat Med eCAM.* (2013) 2013:878521. doi: 10.1155/2013/878521

31. Zhang Y, Yang S, Fan X, Wang S, He N, Li L, et al. Orthogonal design to sift the optimal parameter of neiguan acupuncture for cerebral infarction. *Neural Regen Res.* (2013) 8:2641–8. doi: 10.3969/j.issn.1673-5374.2013.28.006

32. Kim JH, Choi KH, Jang YJ, Bae SS, Shin BC, Choi BT, et al. Electroacupuncture acutely improves cerebral blood flow and attenuates moderate ischemic injury *via* an endothelial mechanism in mice. *PLoS ONE.* (2013) 8:e56736. doi: 10.1371/journal.pone.0056736

33. Chi L, Du K, Liu D, Bo Y, Li W. Electroacupuncture brain protection during ischemic stroke: a role for the parasympathetic nervous system. *J Cereb Blood Flow Metab.* (2018) 38:479–91. doi: 10.1177/0271678X17697988

34. Li J, He J, Du Y, Cui J, Ma Y, Zhang X. Electroacupuncture improves cerebral blood flow and attenuates moderate ischemic injury *via* angiotensin Ii its receptors-mediated mechanism in rats. *BMC Complement Altern Med.* (2014) 14:441. doi: 10.1186/1472-6882-14-441

35. Li J, He Y, Du YH, Zhang M, Georgi R, Kolberg B, et al. Effect of electro-acupuncture on vasomotor symptoms in rats with acute cerebral infarction based on phosphatidylinositol system. *Chin J Integr Med.* (2022) 28:145–52. doi: 10.1007/s11655-021-3341-6

36. Yang B, Zhang C, Feng G, Chen H. The effect of acupuncture on plasma endothelin content in cerebral infarction patients–a clinical study. *J Tradit Chin Med.* (2007) 27:197–8.

37. Li J, Ma Y, Miao XH, Guo JD Li DW. Neovascularization and tissue regeneration by endothelial progenitor cells in ischemic stroke. *Neurol Sci J Italian Neurol Soc Italian Soc Clin Neurophysiol.* (2021) 42:3585–93. doi: 10.1007/s10072-021-05428-3

38. Xie C, Gao X, Luo Y, Pang Y, Li M. Electroacupuncture modulates stromal cell-derived factor-1α expression and mobilization of bone marrow endothelial progenitor cells in focal cerebral ischemia/reperfusion model rats. *Brain Res.* (2016) 1648(Pt A):119–26. doi: 10.1016/j.brainres.2016.07.038

39. Du Y, Shi L, Li J, Xiong J, Li B, Fan X. Angiogenesis and improved cerebral blood flow in the ischemic boundary area were detected after electroacupuncture treatment to rats with ischemic stroke. *Neurol Res.* (2011) 33:101–7. doi: 10.1179/016164110X12714125204317

40. Lee S, Kim W, Park J, Jang HH, Lee SM, Woo JS, et al. Effects of electroacupuncture on endothelial function and circulating endothelial progenitor cells in patients with cerebral infarction. *Clin Exp Pharmacol Physiol.* (2015) 42:822–7. doi: 10.1111/1440-1681.12413

41. Pan J, Zhang W, Yan J, Chang XR, Zhang H, Chen WS, et al. Effects of electroacupuncture of acupoints of pericardium meridian on serum vegf content and cerebral vegf expression in cerebral ischemia rats. *Zhen Ci Yan Jiu.* (2012) 37:197–201.

42. Mao QJ, Chen BG. Effects of Electroacupuncture on microvascular ultrastructure and vegf expression of the right cerebral cortex in focal cerebral ischemia/reperfusion injury rats. *Zhen Ci Yan Jiu.* (2012) 37:476–81.

43. Zheng HZ, Jiang W, Zhao XF, Du J, Liu PG, Chang LD, et al. Electroacupuncture induces acute changes in cerebral cortical mirna profile, improves cerebral blood flow and alleviates neurological deficits in a rat model of stroke. *Neural Regen Res.* (2016) 11:1940–50. doi: 10.4103/1673-5374.197135

44. Zhou Y, Zhang YX, Yang KL, Liu YL, Wu FH, Gao YR, et al. Connexin 43 mediated the angiogenesis of buyang huanwu decoction *via* vascular endothelial growth factor and angiopoietin-1 after ischemic stroke. *Chin J Physiol.* (2022) 65:72–9. doi: 10.4103/cjp.cjp_94_21

45. Xue L, Chen H, Zhang T, Chen J, Geng Z, Zhao Y. Changes in serum vascular endothelial growth factor and endostatin concentrations associated with circulating endothelial progenitor cells after acute ischemic stroke. *Metab Brain Dis.* (2017) 32:641–8. doi: 10.1007/s11011-017-9953-z

46. Ma J, Luo Y. Effects of electroacupuncture on expressions of angiogenesis factors and anti-angiogenesis factors in brain of experimental cerebral ischemic rats after reperfusion. *J Tradit Chin Med.* (2008) 28:217–22. doi: 10.1016/S0254-6272(08)60050-3

47. Li J, Du Y, Zhang X, Bai Z, Pang B, Zhang J, et al. Effects of electroacupuncture on expression of Ang/Tie-2 Mrna and protein in rats with acute cerebral infarction. *J Tradit Chin Med.* (2017) 37:659–66. doi: 10.1016/S0254-6272(17)30320-5

48. Kim DY, Park JA, Kim Y, Noh M, Park S, Lie E, et al. Salm4 regulates angiogenic functions in endothelial cells through Vegfr2 phosphorylation at Tyr1175. *FASEB J Fed Am Soc Experimental Biol.* (2019) 33:9842–57. doi: 10.1096/fj.201802516RR

49. Zan XC, Tang W, Li SL, Gong L, Li MX. Electroacupuncture combined with rehabilitation training improves regional cerebral blood flow and reduces infarct volume by promoting expression of angiogenesis-related factors in acute cerebral ischemia rats. *Zhen Ci Yan Jiu.* (2019) 44:547–53. doi: 10.13702/j.1000-0607.180881

50. Shi S, Wang M, Liu X, Han S, Zhu P. Scalp electroacupuncture promotes angiogenesis after stroke in rats by activation of Wnt/β-Catenin signal pathway. *Evid Based Complement Alternat Med eCAM.* (2022) 2022:1649605. doi: 10.1155/2022/1649605

51. Chang SQ, Zhang XC, Zhang A, Song YY, Jiang SY, Zhang ZH, et al. Acupuncture intervention prolongates thrombolysis time window by regulating cerebral cortex angiogenesis-related genes and proteins in cerebral infarction rats. *Zhen Ci Yan Jiu.* (2021) 46:751–6. doi: 10.13702/j.1000-0607.201121

52. Wu Y, Hu R, Zhong X, Zhang A, Pang B, Sun X, et al. Electric acupuncture treatment promotes angiogenesis in rats with middle cerebral artery occlusion through Ephb4/Ephrinb2 mediated Src/Pi3k signal pathway. *J Stroke Cerebrovasc Dis J Nat Stroke Assoc.* (2021) 30:105165. doi: 10.1016/j.jstrokecerebrovasdis.2020.105165

53. Cui L, Qu H, Xiao T, Zhao M, Jolkkonen J, Zhao C. Stromal cell-derived factor-1 and its receptor Cxcr4 in adult neurogenesis after cerebral ischemia. *Restor Neurol Neurosci.* (2013) 31:239–51. doi: 10.3233/RNN-120271

54. Zhu WK, Shen H, Zhang PX, Zhong L, Zhao YY, Wang CM, et al. Effect of electroacupuncture on expression of stromal cell derived factor-1α in focal ischemic cerebral tissue of rats with cerebral ischemia/ reperfusion injury. *Zhen Ci Yan Jiu.* (2019) 44:649–52. doi: 10.13702/j.1000-0607.180429

55. Han L, Li J, Chen Y, Zhang M, Qian L, Chen Y, et al. Human urinary kallidinogenase promotes angiogenesis and cerebral perfusion in experimental stroke. *PLoS ONE.* (2015) 10:e0134543. doi: 10.1371/journal.pone.0134543

56. Yang LH, Du YH Li J. Effect of electroacupuncture on expression of Apelin-Apj system of cerebral vascular endothelial cell in rats with cerebral infarction. *Zhen Ci Yan Jiu.* (2017) 42:9–13.

57. Yang L, Yang BC, Zhang CX, Tong J. Protective effects of acupuncture and lgnhfd on expressions of vascular endothelial growth factor, basic fibroblast growth factor, and cluster of differentiation 34 in rats with cerebral ischemia-reperfusion injury. *J Tradit Chin Med.* (2021) 41:463–70. doi: 10.19852/j.cnki.jtcm.2021.03.015

58. Shi L, Cao HM Li Y, Xu SX, Zhang Y, Zhang Y, et al. Electroacupuncture improves neurovascular unit reconstruction by promoting collateral circulation and angiogenesis. *Neural Regen Res.* (2017) 12:2000–6. doi: 10.4103/1673-5374.221156

59. Carver KA, Lourim D, Tryba AK, Harder DR. Rhythmic expression of cytochrome P450 epoxygenases Cyp4x1 and Cyp2c11 in the rat brain and vasculature. *Am J Physiol Cell Physiol.* (2014) 307:C989–98. doi: 10.1152/ajpcell.00401.2013

60. Lu Y, Zhao HJ, Wang Y, Han BB, Wang T, Sun Z, et al. Effect of electroacupuncture intervention on neurological function, cerebral blood flow and cerebral cytochrome P 450 2 C 11 Mrna expression in rats with focal cerebral ischemia. *Zhen Ci Yan Jiu.* (2014) 39:345–50.

61. Zhang JQ, Meng ZH, Fan XN. Efficacy judgment for treating ischemic stroke by acupuncturing at neiguan (Pc6) and the screening for optimal parameters. *Zhongguo Zhong Xi Yi Jie He Za Zhi.* (2013) 33:526–30.

62. Wang Y, Zhang XB, Xiao W, Wang Z, Kong HB, Wang J, et al. Effect of nape cluster-acupuncture treatment on brain microcirculation in ischemic stroke rats. *Zhen Ci Yan Jiu.* (2013) 38:220–3.

63. Zhou F, Guo J, Cheng J, Wu G, Xia Y. Electroacupuncture increased cerebral blood flow and reduced ischemic brain injury: dependence on stimulation intensity and frequency. *J Appl Physiol.* (2011) 111:1877–87. doi: 10.1152/japplphysiol.00313.2011

64. Borsody MK, Yamada C, Bielawski D, Heaton T, Castro Prado F, Garcia A, et al. Effects of non-invasive facial nerve stimulation in the dog middle cerebral artery occlusion model of ischemic stroke. *Stroke.* (2014) 45:1102–7. doi: 10.1161/STROKEAHA.113.003243

65. Chiluwal A, Narayan RK, Chaung W, Mehan N, Wang P, Bouton CE, et al. Neuroprotective effects of trigeminal nerve stimulation in severe traumatic brain injury. *Sci Rep.* (2017) 7:6792. doi: 10.1038/s41598-017-07219-3

66. Levi H, Schoknecht K, Prager O, Chassidim Y, Weissberg I, Serlin Y, et al. Stimulation of the sphenopalatine ganglion induces reperfusion and blood-brain barrier protection in the photothrombotic stroke model. *PLoS ONE.* (2012) 7:e39636. doi: 10.1371/journal.pone.0039636

67. Gong Y, Li N, Lv Z, Zhang K, Zhang Y, Yang T, et al. The neuro-immune microenvironment of acupoints-initiation of acupuncture effectiveness. *J Leukoc Biol.* (2020) 108:189–98. doi: 10.1002/JLB.3AB0420-361RR

68. Liu JT, Lee JK, Chang PJ, Sun CM. Increased regional cerebral perfusion in contralateral motor and somatosensory areas after median nerve stimulation therapy. *Acta Neurochirurgica Supp.* (2008) 101:65–70. doi: 10.1007/978-3-211-78205-7_11

69. Suzuki T, Waki H, Imai K, Hisajima T. Electroacupuncture of the ophthalmic branch of the trigeminal nerve: effects on prefrontal cortex blood flow. *Med Acupunct.* (2020) 32:143–9. doi: 10.1089/acu.2019.1406

70. Hennerici MG, Kern R, Szabo K. Non-pharmacological strategies for the treatment of acute ischaemic stroke. *Lancet Neurol.* (2013) 12:572–84. doi: 10.1016/S1474-4422(13)70091-7

71. Gursoy-Ozdemir Y, Yemisci M, Dalkara T. Microvascular protection is essential for successful neuroprotection in stroke. *J Neurochem.* (2012) 123(Suppl 2):2–11. doi: 10.1111/j.1471-4159.2012.07938.x

72. Przykaza Ł. Understanding the connection between common stroke comorbidities, their associated inflammation, and the course of the cerebral ischemia/reperfusion cascade. *Front Immunol.* (2021) 12:782569. doi: 10.3389/fimmu.2021.782569

73. Chen CH, Hsieh CL. Effect of acupuncture on oxidative stress induced by cerebral ischemia-reperfusion injury. *Antioxidants.* (2020) 9:248. doi: 10.3390/antiox9030248

74. Long M, Wang Z, Zheng D, Chen J, Tao W, Wang L, et al. Electroacupuncture pretreatment elicits neuroprotection against cerebral ischemia-reperfusion injury in rats associated with transient receptor potential vanilloid 1-mediated anti-oxidant stress and anti-inflammation. *Inflammation.* (2019) 42:1777–87. doi: 10.1007/s10753-019-01040-y

75. Sun X, Liu H, Sun Z, Zhang B, Wang X, Liu T, et al. Acupuncture protects against cerebral ischemia-reperfusion injury *via* suppressing endoplasmic reticulum stress-mediated autophagy and apoptosis. *Mol Med.* (2020) 26:105. doi: 10.1186/s10020-020-00236-5

76. Hossmann KA. The two pathophysiologies of focal brain ischemia: implications for translational stroke research. *J Cereb Blood Flow Metab.* (2012) 32:1310–6. doi: 10.1038/jcbfm.2011.186

frontiers | Frontiers in Neurology

TYPE Clinical Trial
PUBLISHED 10 November 2022
DOI 10.3389/fneur.2022.1025269

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Lu Liu,
Capital Medical University, China
Daniela Rodrigues Recchia,
Universität Witten/Herdecke, Germany

*CORRESPONDENCE
Seungwon Kwon
kkokkottung@hanmail.net

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 22 August 2022
ACCEPTED 18 October 2022
PUBLISHED 10 November 2022

CITATION
Jin C, Cho K-H, Kwon S, Lee H-G, Kim T-H, Jung W-S, Moon S-K, Cho S-Y, Kang B-K, Park J-M, Park H-J and Ko C-N (2022) Effectiveness and safety of herbal medicine Ukgansan for clinical symptoms in Parkinson's disease: A pilot, randomized, assessor-blinded clinical trial. *Front. Neurol.* 13:1025269. doi: 10.3389/fneur.2022.1025269

Effectiveness and safety of herbal medicine Ukgansan for clinical symptoms in Parkinson's disease: A pilot, randomized, assessor-blinded clinical trial

Chul Jin[1†], Ki-Ho Cho[2†], Seungwon Kwon[2*], Han-Gyul Lee[2], Tae-Hun Kim[3], Woo-Sang Jung[2], Sang-Kwan Moon[2], Seung-Yeon Cho[4], Byoung-Kab Kang[5], Jung-Mi Park[4], Hi-Joon Park[6] and Chang-Nam Ko[4]

[1]Department of Korean Medicine Cardiology and Neurology, Graduate School, Kyung Hee University, Seoul, South Korea, [2]Department of Cardiology and Neurology, College of Korean Medicine, Kyung Hee University, Kyung Hee University Medical Center, Seoul, South Korea, [3]Korean Medicine Clinical Trial Center, Korean Medicine Hospital, Kyung Hee University, Seoul, South Korea, [4]Department of Cardiology and Neurology, College of Korean Medicine, Kyung Hee University, Kyung Hee University Hospital at Gangdong, Seoul, South Korea, [5]KM Science Research Division, Korea Institute of Oriental Medicine, Daejeon, South Korea, [6]Acupuncture and Meridian Science Research Center, College of Korean Medicine, Kyung Hee University, Seoul, South Korea

Objectives: Parkinson's disease (PD) is a neurodegenerative disease in which patients are suffering various symptoms. Previous experimental studies suggested that herbal medicine Ukgansan (UGS) could be beneficial for PD. The aim of this pilot clinical trial was to evaluate the efficacy of UGS for improving clinical symptoms in patients with PD.

Methods: Sixty patients with idiopathic PD were randomly assigned to receive either UGS plus acupuncture or acupuncture alone for 6 weeks. During the trial, all anti-parkinsonian medications were maintained. Subjects were evaluated for various clinical assessments of PD, including the Movement Disorder Society-Sponsored Revision of the Unified PD Rating Scale (MDS-UPDRS) and the 39-item Parkinson's Disease Questionnaire (PDQ-39), until 12 weeks.

Results: In MDS-UPDRS between the groups, no significant time x group interaction was found. In the subgroup analysis of participants with anxiety, a significant time x group interaction was found in the PDQ-39 domain of mobility ($P = 0.007$), activities of daily living ($P = 0.042$), and the PDQ-39 summary index ($P = 0.048$). In addition, *post-hoc* analysis in participants with anxiety showed a significant decrease in the domains of mobility ($P = 0.001$) and activities of daily living ($P = 0.013$) at week 7. There were no adverse events associated with UGS.

Conclusion: The additional administration of UGS has the potential to significantly improve the quality of life of PD patients with anxiety. In order to create more definitive evidence, clinical trials with more rigorous methodologies should be conducted in future.

Clinical trial registration: http://cris.nih.go.kr, identifier: KCT0003444.

KEYWORDS

Parkinson's disease, Ukgansan, adjunctive therapy, anxiety, quality of life, the 39-item Parkinson's Disease Questionnaire

Introduction

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder, with a prevalence of 2–3% in the elderly population (1). In 2015, 6.2 million people worldwide were diagnosed with PD, and the number is projected to be over 12 million by 2040 (2).

Parkinson's disease (PD) is characterized by a lack of dopamine within basal ganglia due to cell loss of dopaminergic neurons in the substantia nigra, resulting in motor symptoms such as bradykinesia, resting tremor, rigidity, and postural instability. Therefore, the gold-standard treatment of PD is to alleviate parkinsonian symptoms with drugs that increase dopamine concentrations (levodopa) or directly stimulate dopamine receptors (dopamine agonists) (3). However, long-term use of anti-PD medications reduces their effectiveness and causes side effects such as neuropsychiatric symptoms and motor complications. In addition, PD patients are suffered from not only motor symptoms, but also numerous non-motor symptoms, such as depression, fatigue, sleep disorder, cognitive impairment, autonomic dysfunction, and pain which might start in the prodromal phase of PD (1, 3). Thus, managing these various symptoms of PD requires a variety of therapeutic options in addition to conventional therapy.

Complementary alternative medicine (CAM) treatments for PD include meditation, qigong, yoga, dance, massage, acupuncture, and herbal medicine (4). Among them, acupuncture has been used as an effective therapeutic option in patients with PD in eastern Asia (5). Previous clinical trials have shown that acupuncture has the clinical efficacy to improve motor symptoms and some non-motor symptoms in patients with PD (6). Furthermore, acupuncture has also been found to protect dopaminergic neurons from degeneration by altering the neurotransmitter balance in the basal ganglia circuit and reducing oxidative stress, inflammation, and apoptosis (7).

As another option, that herbal medicine as an adjunct therapy can help improve motor and non-motor symptoms of PD (8, 9). Ukgansan (UGS, Yokukansan in Japanese, Yigansan in Chinese) is an herbal medicine formula that may benefit the treatment of PD and that has been shown to protect dopaminergic neurons and to supplement dopamine concentration (10, 11). However, the therapeutic role of UGS in patients with PD is debatable. UGS has improved neuropsychiatric symptoms but not motor function in clinical trials for PD (12, 13). Therefore, an exploratory pilot clinical trial to determine whether UGS helps improve clinical symptoms of PD is needed. The present pilot study explored the efficacy and safety of UGS for improving clinical symptoms in patients with PD.

Materials and methods

Study design

This study was a single-centered, randomized controlled, assessor-blinded, active-controlled, parallel-group, pilot clinical trial conducted at the Kyung Hee University Korean Medicine Hospital (Seoul, Republic of Korea) between December 2018 and November 2020. The protocol was registered with the Clinical Research Information Service (CRIS, KCT0003444) and approved by the Institutional Review Board of Kyung Hee University Korean Medicine Hospital (KOMCIRB-170717-HR-02).

Participants were recruited through advertisements, which are poster ads on bulletin boards of the hospital and the subway. This pilot trial had three consecutive periods: screening period (week 0), intervention period (weeks 1–6), and observation period (weeks 7–12). All prospective participants were questioned by phone by the clinical research coordinator (CRC) to check their medications and PD duration before being scheduled for a screening visit (Visit 0). At the screening visit, patients who voluntarily agreed to participate in the clinical trial were evaluated on the inclusion and exclusion criteria, including blood sampling and electrocardiogram (ECG).

At week 1, baseline measurements were taken on eligible participants. After that, they were randomly assigned to one of two groups, the control group (only acupuncture + medications related to Parkinson's disease) or the treatment group (UGS + acupuncture + medications related to Parkinson's disease) in a 1:1 ratio. From that day on, participants in both groups maintained medications related to Parkinson's disease and were treated for acupuncture two times a week for 6 weeks. The treatment group was also prescribed UGS for the same period. Measurements for evaluations were taken at week 7 and week 12 (Figure 1).

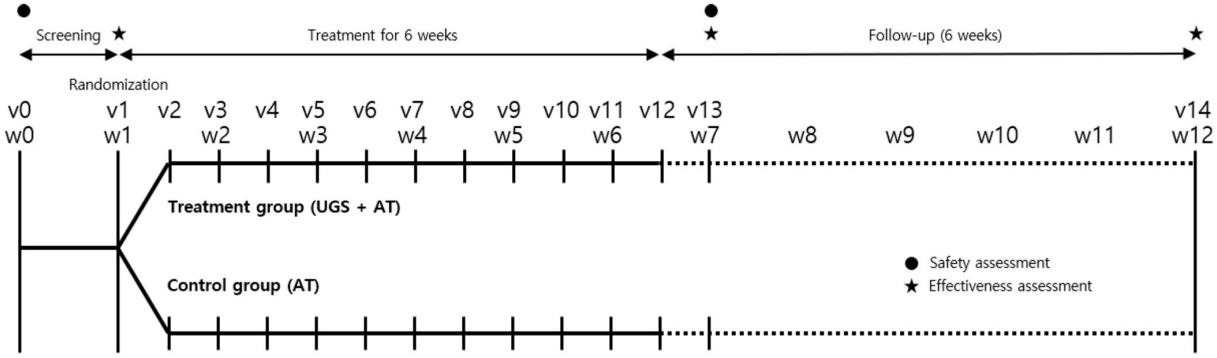


FIGURE 1
Timeline of study. The vertical lines indicate the time points of the actual visit for trial, and the dotted lines show the observation period after the intervention. Clinical effectiveness was assessed with the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), the modified Hoehn and Yahr staging scale (H–Y scale), Schwab and England Activities of Daily Living (SE-ADL) Scale, Berg balance test (BBT), Timed Up and Go test (TUG), 39-item Parkinson's Disease Questionnaire (PDQ-39), and five-level version of European Quality of Life Scale Five-Dimensional Questionnaire (EQ-5D-5L) score. Self-reported adverse events and measured vital signs were evaluated at each visit. Electrocardiogram and blood sampling were performed before and after treatment for safety assessment. v, visit; w, week; UGS, Ukgansan; AT, acupuncture.

If the following situation occurred while participating in the study, it was treated as a withdrawal:

1. Violation of the inclusion or exclusion criteria after enrollment,
2. Withdrawal of consent by the participants (or their legal representative),
3. Request for discontinuance of the trial or refusal to receive intervention by the participants (or their legal representative),
4. Loss to follow-up,
5. Serious adverse events (e.g., hospitalization or life-threatening events, etc.) or exacerbation of illnesses that made it difficult to continuous participation,
6. Less than 80% compliance of clinical trial medication (UGS) administration and/or acupuncture treatment,
7. Violation of the clinical trial protocol,
8. Other inappropriate conditions for study participation as judged by the investigator.

Participants

Participants who met the following criteria were selected as subjects of the present study.

Inclusion criteria

1. Males or females aged 45–80 years,
2. Patients with idiopathic Parkinson's disease taking levodopa for more than 5 years (without anti-cholinergic drugs),
3. Hoehn and Yahr stages 2–3,
4. Patients without cognitive impairment who have voluntarily agreed to participate.

Exclusion criteria

1. Patients diagnosed with secondary parkinsonism or atypical parkinsonian syndrome,
2. Diseases that might affect the administration or absorption of drugs (e.g., dysphagia, clinically severe digestive disorders, galactose intolerance, Lapp lactase deficiency, or glucose–galactose malabsorption),
3. Previous history of severe heart disease (myocardial infarction, heart failure, etc.),
4. Patients receiving neurosurgical treatment (e.g., deep brain stimulation),
5. Patients with glaucoma,
6. Patients who were diagnosed or treated for cancer within 5 years,
7. Chronic alcohol consumption or drug abuse,
8. Patients with a history of allergy to the test drug (UGS),
9. Patients with liver disease or kidney disease (aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), or creatinine >x3 of normal upper limit),
10. Women who were planning pregnancy, pregnant, or breastfeeding,
11. Participation in other clinical trials within 30 days,
12. Patients who were judged by the investigator to be unsuitable for participation in the trial due to psychiatric symptoms, medical illness, laboratory findings, etc.

Interventions

All participants maintained medications related to Parkinson's disease (levodopa, dopamine agonists, monoamine oxidase B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, amantadine, etc.) except for anti-cholinergic drugs, which had been taken as before.

Control group

The acupuncture therapy was performed two times a week for 6 weeks on participants in the control arm. Acupuncture was conducted by a Korean medical doctor with more than 7 years of clinical expertise in internal medicine. The STRICTA checklist included instructions on how to practice acupuncture (Table 1) (14).

Treatment group

Participants in the treatment group received the same acupuncture procedure as the control group and additional administration of UGS. UGS, an herbal extract granule, was produced and packaged by KYUNGJIN PHARM.CO.LTD. (Icheon, South Korea), one of the Korean Good Manufacturing Practice (KGMP)-certified companies, for this clinical trial under the approval of the Ministry of Food and Drug Safety's Investigational New Drug (IND). The composition of UGS is described in Table 2. At visits 1, 5, and 9, participants were provided with individually packed UGS (14 days + an extra 2 days). UGS was provided by an independent pharmacist in a separate place, and participants were required to take that medicine with water at 10 a.m. and 5 p.m. for 6 weeks.

Outcomes

Primary outcome

The primary outcome of this study was a change in the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) total score after treatment (week 7 minus baseline), between the two groups (15).

Secondary outcomes

The secondary outcomes included MDS-UPDRS part I–IV, trends of changes in MDS-UPDRS, the modified Hoehn and Yahr staging scale (H–Y scale), Schwab and England Activities of Daily Living (SE-ADL) Scale, Berg balance test (BBT), Timed-up-and-go (TUG) test, 39-item Parkinson's Disease Questionnaire (PDQ-39) including the PDQ-39 summary index (PDQ-39 SI), and five-level version of European Quality of Life Scale Five-Dimensional Questionnaire (EQ-5D-5L) score (16, 17). All these measured outcomes were compared between the two groups with the changes in difference from baseline.

TABLE 1 Revised STRICTA* checklist.

1. Acupuncture rationale	1a) Manual acupuncture
	1b) Based on a systematic review[5] and expert consensus
	1c) Unacceptable
2. Details of needling	2a) 23 points
	2b) GV20, bilateral EX-HN5, GB20, LI4, TE5, LI10, LI11, GB34, ST36, GB39, LR3, GB41†
	2c) 5-10mm
	2d) De-qi
	2e) Manual stimulation
	2f) 20 minutes
	2g) Sterilized stainless steel needle (diameter 0.25mm, length 40mm, Dongbang Medical co., Seongnam, South Korea)
3. Treatment regimen	3a) 12 sessions
	3b) two times a week for 6 weeks
4. Other components of treatment	4a) The purpose of this study was to verify the additional effects of UGS when used in conjunction with acupuncture. As a result, during the intervention time, the treatment group received UGS two times a day, and the anti-Parkinson's medicine remained the same.
	4b) The study was conducted in an outpatient treatment room at a single Korean medicine hospital. Before deciding to enroll in the clinical study, the patient was given all pertinent information.
5. Practitioner background	5) All acupuncture procedures were performed by one Korean Medicine Doctor with more than 7 years of clinical experience.
6. Control interventions	6a) Based on the systematic review[5] and expert consensus
	6b) Same as acupuncture procedure of the treatment group

*Standards for Reporting Interventions in Clinical Trials of Acupuncture.
†The terminology was followed as the WHO Standard Acupuncture Point Locations in the Western Pacific Region.

Safety assessment

Vital signs and adverse events were measured at each visit. ECG and blood sampling were performed at the screening visit and week 7. Blood sampling includes the white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), platelet count, BUN, creatinine, AST, ALT, glucose, and electrolytes (Na, K, Cl).

Sample size calculation

This clinical trial was to verify the additional improvement in MDS-UPDRS total score following the administration of

TABLE 2 Composition of Ukgansan*.

Scientific name	Herbal name	Amount (*g*)
Atractylodes lancea De Candolle	Atractylodis Rhizoma	4
Poria cocos Wolf	Poria Sclerotium	4
Magnolia officinalis Rehder et Wilson	Magnoliae Cortex	4
Poncirus trifoliata Rafinesque	Ponciri Fructus Immaturus	4
Cnidium officinale Makino	Cnidii Rhizome	3
Uncaria sinensis (Oli.) Havil	Uncariae Ramulus Et Uncus	3
Angelica gigas Nakai	Angelicae Gigantis Radix	3
Bupleurum falcatum Linné	Bupleuri Radix	2
Glycyrrhiza uralensis Fisher	Glycyrrhizae Radix et Rhizoma	1.5

*The Ukgansan used in this study was modified by adding Magnoliae Cortex and Ponciri Fructus Immaturus to original Ukgansan.

herbal medicine UGS. The effect size was calculated from the previous study which was conducted to validate the effect of herbal medicine and performed by using original UPDRS (18). The pooled standard deviation of the UPDRS total score at post-treatment in a previous similar study was 16.64. And a minimal clinical important difference (CID) on the UPDRS total score was 4.3 points (19). Assuming the mean difference of 4.3 and the standard deviation of 16.64, the calculated effect size was 0.258. We calculated the sample size using stepped rules of thumb for pilot sample size, using a two-tailed type I error rate of 5% and a power of 90%. As a result, each group required 25 participants (20). Sixty participants, 30 in each group, were recruited, anticipating a 15% drop-out rate similar to the previous study (21).

Randomization, allocation concealment, and blinding

An independent statistician generated random numbers by block randomization using STATA (version 4.2; StataCorp LLC, Texas, USA). The random assignment code was placed in opaque sealed envelopes and delivered to the Kyung Hee University Korean Medicine Hospital. The investigator opened the consecutive numbered sealed envelope in front of the participant whose screening criteria were satisfied and assigned them to the intervention or control group. All opened envelopes were kept safely and separately.

The design of this study does not include placebo as a control; therefore, the participants and practitioners cannot be blinded. However, the separate assessor who was responsible for evaluating efficacy and safety was blinded to prevent access to the allocation results.

Statistical analysis

At baseline assessments, continuous data were expressed as means and standard deviations and analyzed with either the two-sample *t*-test or the Wilcoxon rank-sum test. Categorical variables were represented as frequencies or percentages, and the Chi-square test or Fisher's exact test was used to assess them.

The effectiveness analysis was preferentially performed using the full analysis set (FAS), including participants who had been treated more than once after participating in the clinical trial. If necessary, the per-protocol set (PPS) was performed, including participants who had completed the clinical trial plan. In the FAS analysis, missing data were handled with the last-observation-carried-forward (LOCF) analysis imputation method.

The MDS-UPDRS score changes in primary and secondary outcomes, between baseline (before the randomization) and week 7 (1 week after 6 weeks of treatment), were compared between the two groups using either the two-sample *t*-test or the Wilcoxon rank-sum test. A repeated measures analysis of variance (ANOVA) was used to examine the trend of changes in the MDS-UPDRS score between groups depending on the time. The modified H–Y scale evaluated at two endpoints was classified as an improvement, no change, or aggravation, compared with baseline. And the improvement ratio between groups was conducted using the Chi-square test or Fisher's exact test. The other secondary outcomes, comparing the change scores between groups at weeks 7 and 12, were analyzed using the same methods as the primary outcome. The significance of the statistical values between groups at each time point was evaluated using an independent *t*-test as a *post-hoc* analysis.

The safety of treatment was assessed using the safety analysis set (SAS) method, which included all subjects who had been treated at least once. And the Chi-square test or Fisher's exact test was used whether there were any differences in the incidence ratio between the groups.

This pilot clinical trial was conducted with a two-sided test to determine significance at the 5% level ($\alpha = 0.05$). In the *post-hoc* test, the Bonferroni correction was applied to test the significance (statistically significant when $p < 0.016$). All statistical analyses were performed using the IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics

A total of 64 patients were screened for eligibility. Of these 64, three did not meet the inclusion criteria, one

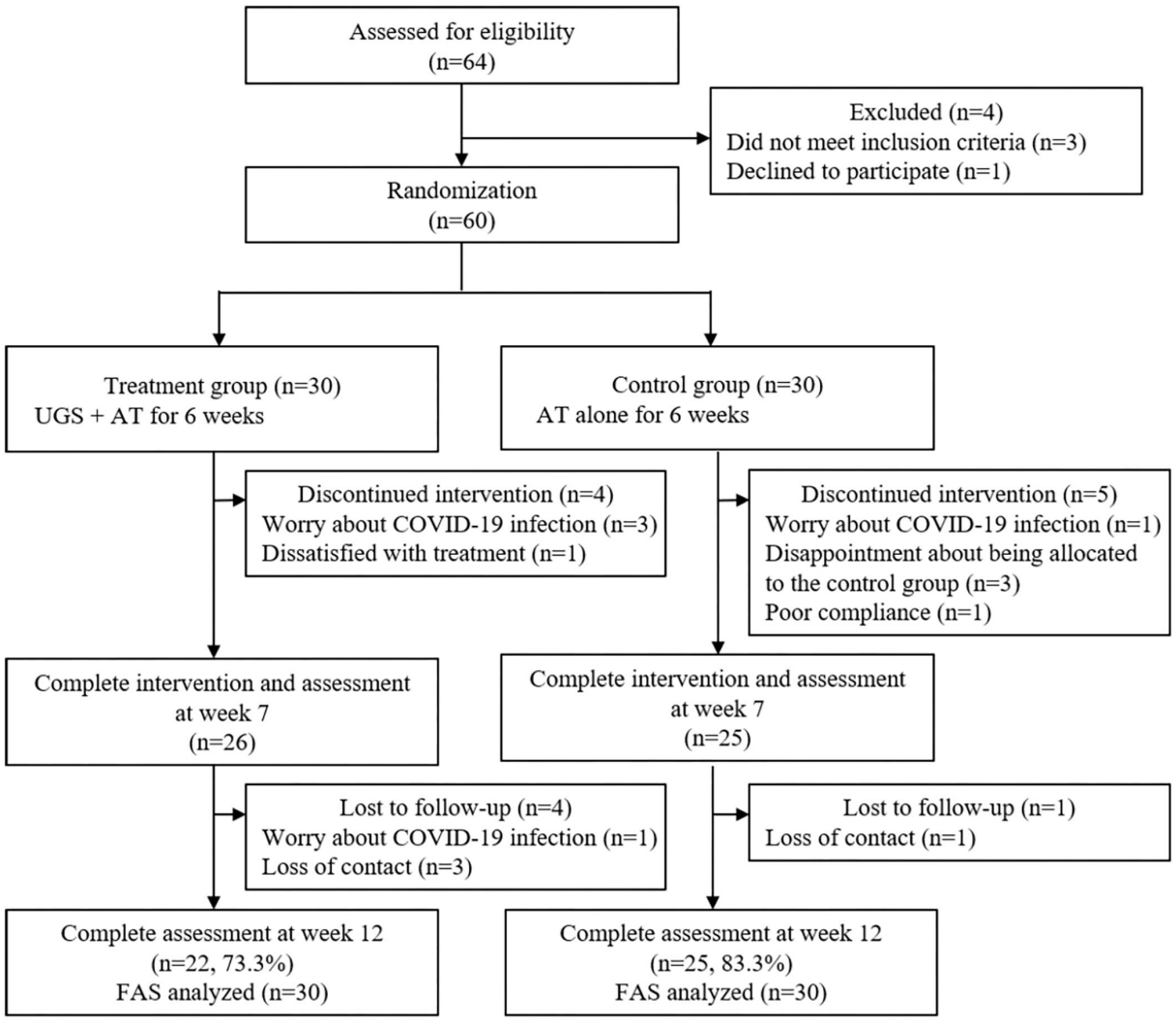


FIGURE 2
Study flow diagram. UGS, Ukgansan; AT, acupuncture; COVID-19, coronavirus disease 2019.

withdrew one's consent, and the remaining 60 subjects were randomly assigned to the treatment group and the control group, 30 in each group. During the 6-week treatment period, four participants withdrew from the treatment group, and five dropped out of the control group. And four patients in the treatment group and one in the control group did not show up for a follow-up visit after 6 weeks. As a result, 22 (73.3%) of the treatment participants and 25 (83.3%) of the control participants completed the final evaluation (Figure 2).

The demographic and baseline characteristics of both groups are listed in Table 3. There were no statistically significant differences in demographics, clinical features, or outcome variables.

MDS-UPDRS

At the repeated measures analysis of variance used to determine the trend of change in MDS-UPDRS between the groups, no significant time x group interaction was found. In the total MDS-UPDRS score and part III total score, however, only the time effect was significant, indicating that the two scores tended to decrease until the 12-week observation period (Table 4).

In both groups, the MDS-UPDRS total score and MDS-UPDRS part I–IV score measured at week 7 decreased from baseline. However, the difference in total MDS-UPDRS score from baseline at week 7, which was a primary outcome, did not show any significance between the two groups (95% CI: −0.82 to

TABLE 3 Demographic and baseline characteristics.

Characteristics	Treatment group ($n = 30$)	Control group ($n = 30$)	*P* value
Age, mean (SD), years	66.87 (8.04)	64.27 (7.18)	0.192
Male, n (%)	15 (50.00)	19 (63.33)	0.297
BMI, mean (SD), kg/m^2	22.51 (2.56)	23.92 (2.96)	0.053
Employment status			
Employed, *n* (%)	3 (10.00)	1 (3.33)	
Unemployed, *n* (%)	27 (90.00)	29 (96.67)	0.612
Education			
Primary school, *n* (%)	17 (56.67)	8 (26.67)	
Middle school, *n* (%)	7 (23.33)	13 (43.33)	
High school, *n* (%)	3 (10.00)	7 (23.33)	
College or higher, *n* (%)	3 (10.00)	2 (6.67)	0.074
PD history, mean (SD), years	9.27 (3.77)	9.10 (3.36)	0.857
Levodopa dosage, mean (SD), mg/day	626.67 (303.35)	691.67 (274.99)	0.336
MDS-UPDRS total score, mean (SD)	57.47 (20.35)	58.63 (26.67)	0.850
MDS-UPDRS part I, mean (SD)	12.40 (6.08)	13.53 (7.82)	0.533
MDS-UPDRS part II, mean (SD)	14.37 (7.54)	15.10 (8.47)	0.724
MDS-UPDRS part III, mean (SD)	27.77 (13.71)	26.07 (14.12)	0.638
MDS-UPDRS part IV, mean (SD)	2.93 (3.34)	3.93 (3.84)	0.286
Modified Hoehn–Yahr stage			
Stage 2, *n* (%)	25 (83.33)	23 (76.67)	
Stage 2.5, *n* (%)	1 (3.33)	2 (6.67)	
Stage 3, *n* (%)	4 (13.33)	5 (16.67)	0.794
BBT, mean (SD)	53.00 (3.24)	51.73 (4.95)	0.245
TUG, mean (SD), sec	8.56 (2.80)	9.49 (6.58)	0.484
SE-ADL, mean (SD), %	82.00 (10.64)	83.00 (12.08)	0.735
Domains of PDQ-39, mean (SD), %			
Mobility	27.58 (19.78)	29.17 (23.22)	0.777
Activities of Daily Living	29.31 (19.50)	25.56 (24.34)	0.513
Emotional well-being	29.86 (23.85)	31.67 (24.29)	0.772
Stigma	24.17 (16.47)	28.96 (23.81)	0.369
Social support	27.22 (22.52)	20.28 (19.41)	0.206
Cognition	29.79 (21.88)	25.63 (21.17)	0.457
Communication	23.61 (19.34)	23.06 (24.04)	0.922
Bodily discomfort	28.89 (23.03)	31.94 (21.23)	0.595
PDQ-39 SI, mean (SD), %	27.55 (16.11)	27.03 (19.32)	0.910
EQ-5D Index score, mean (SD)	0.72 (0.11)	0.70 (0.15)	0.440
EQ-5D VAS, mean (SD), mm	71.70 (13.75)	73.23 (16.38)	0.696

BMI, body mass index; PD, Parkinson's disease; MDS-UPDRS, the Movement Disorder Society-Unified Parkinson's Disease Rating Scale; BBT, Berg balance test; TUG, Timed Up and Go test; SE-ADL, Schwab and England Activities of Daily Living Scale; PDQ-39, 39-item Parkinson's Disease Questionnaire; PDQ-39 SI, PDQ-39 summary index; EQ-5D, five-level version of European Quality of Life Scale Five-Dimensional Questionnaire; VAS, Visual analog scale.
P value was calculated using independent *t*-test or Wilcoxon rank-sum test.

6.22; $P = 0.801$). Similarly, there were no significant differences in MDS-UPDRS part I–IV scores between the two groups after treatment (Table 5).

H–Y scale

At the two outcome points, weeks 7 and 12, no significant difference in H–Y scale changes was observed between the two groups ($P = 1.00$ and $P = 0.240$, respectively) (Table 6).

Other outcomes (BBT, TUG, SE-ADL, PDQ-39, and EQ-5D-5L)

There were no significant interaction effects evaluated by repeated measures ANOVA in all measured outcomes. Between the two groups, there were no significant differences in the BBT, TUG, SE-ADL, PDQ-39 SI, PDQ-39 domains, EQ-5D index score, and EQ-5D VAS (Table 7).

PDQ-39 domains and summary index in anxiety group

All subjects who answered no to item 21 (anxious) on the PDQ-39 were eliminated, and only those with anxiety were subjected to subgroup analysis. In a subgroup of anxiety, *post-hoc* analysis was performed on the PDQ-39. In this subgroup analysis, Cronbach's alpha value for PDQ-39 was 0.926, which showed high reliability.

In a repeated measures ANOVA to investigate changes between the two anxiety groups over time, the PDQ-39 SI score, mobility domain, and activities of daily living domain showed a significant time x group interaction effect ($P = 0.048$, $P = 0.007$, and $P = 0.042$, respectively, Table 8). And comparing the differences from baseline between the two groups, the treatment group with anxiety showed a significant improvement in the domains of mobility and activities of daily living compared with the control group with anxiety at week 7 ($P = 0.001$, $P = 0.013$, respectively, Table 8).

Safety

For the safety analysis, a total of 60 subjects were included. There were no serious adverse events in either group, and the trial was not terminated due to any adverse events. A subject in the treatment group reported mild dizziness ($n = 1$, 3.33 %). After acupuncture treatment, that participant experienced mild dizziness, which went away after a brief rest. At week 7, a subject in the control group ($n = 1$, 3.33 %) reported an increase in

TABLE 4 The trend of changes during 12 weeks in the MDS-UPDRS score between groups depending on the time using repeated measures analysis of variance.

Variable	Time	Treatment group ($n = 30$)	Control group ($n = 30$)	Source	F value	*P* value
Total MDS-UPDRS score	Baseline	57.47 ± 20.35	58.63 ± 26.67	Group	0.008	0.930
	Week 7	53.03 ± 18.55	53.30 ± 24.01	Time	8.406	0.001
	Week 12	52.13 ± 21.89	49.23 ± 25.20	Time x group	0.683	0.489
MDS-UPDRS Part I score	Baseline	12.40 ± 6.08	13.53 ± 7.82	Group	0.169	0.682
	Week 7	11.90 ± 5.64	12.30 ± 6.57	Time	2.076	0.138
	Week 12	11.63 ± 4.82	11.93 ± 6.75	Time x group	0.286	0.717
MDS-UPDRS Part II score	Baseline	14.37 ± 7.54	15.10 ± 8.47	Group	0.217	0.643
	Week 7	13.60 ± 6.20	15.00 ± 9.29	Time	0.225	0.799
	Week 12	14.07 ± 8.06	14.57 ± 8.12	Time x group	0.204	0.816
MDS-UPDRS Part III score	Baseline	27.77 ± 13.71	26.07 ± 14.12	Group	0.777	0.382
	Week 7	24.80 ± 15.05	22.13 ± 12.58	Time	10.889	<0.001
	Week 12	23.97 ± 14.17	19.50 ± 13.62	Time x group	0.771	0.447
MDS-UPDRS Part IV score	Baseline	2.93 ± 3.34	3.93 ± 3.84	Group	1.274	0.264
	Week 7	2.73 ± 3.56	3.87 ± 4.13	Time	2.025	0.137
	Week 12	2.47 ± 2.78	3.23 ± 3.71	Time x group	0.187	0.830

MDS-UPDRS, the Movement Disorder Society-Unified Parkinson's Disease Rating Scale.
Values are presented as mean ± standard deviation.

TABLE 5 The difference in MDS-UPDRS scores between groups from baseline to week 7.

	Treatment group ($n = 30$)	Control group ($n = 30$)	Mean difference	95% confidence interval (CI)	*P* value	Effect size
Total MDS-UPDRS score	−4.43 ± 14.04	−5.33 ± 13.50	−0.90	−8.02 to 6.22	0.801	0.065
Part I score (non–motor symptom)	−0.50 ± 4.97	−1.23 ± 5.07	–	–	0.868	0.145
Part II score (motor symptom)	−0.77 ± 5.79	−0.10 ± 5.17	–	–	0.747	0.122
Part III score (motor examination)	−2.97 ± 8.53	−3.93 ± 8.75	−0.97	−5.43 to 3.50	0.666	0.111
Part IV score (motor complications)	−0.20 ± 2.62	−0.07 ± 2.53	–	–	0.760	0.050

MDS-UPDRS, the Movement Disorder Society-Unified Parkinson's Disease Rating Scale.
Values are presented as mean ± standard deviation.
P value was calculated using independent *t*-test or Wilcoxon rank-sum test.
Effect size was calculated using Cohen's D.

TABLE 6 Comparison of H–Y scale improvement rates between groups.

	Change of H–Y scale	Treatment group	Control group	*P* value
		n (%)		
Week 7–baseline	Improvement	10 (33.3)	10 (33.3)	1.00
	No change	19 (63.3)	20 (66.7)	
	Aggravation	1 (3.3)	0 (0)	
Week 12–baseline	Improvement	12 (40.0)	18 (60.0)	0.240
	No change	17 (56.7)	11 (36.7)	
	Aggravation	1 (3.3)	1 (3.3)	

H–Y scale, Modified Hoehn–Yahr stage scale.
P-value was calculated by Fisher's exact test.

TABLE 7 Comparison of other outcomes between two groups.

	Week	Treatment group ($n = 30$)	Control group ($n = 30$)	Source	*P* value*	*P* value†
BBT	0	53.00 ± 3.24	52.07 ± 4.59	Group	0.665	–
	7	53.27 ± 2.97	53.33 ± 4.33	Time	0.007	0.071
	12	53.63 ± 3.31	53.27 ± 4.54	T × G	0.236	0.374
TUG	0	8.56 ± 2.80	9.49 ± 6.58	Group	0.521	–
	7	7.71 ± 2.56	8.55 ± 6.56	Time	<0.001	0.800
	12	7.90 ± 2.89	8.63 ± 6.66	T × G	0.844	0.985
SE-ADL	0	82.00 ± 10.64	83.00 ± 12.08	Group	0.680	–
	7	84.00 ± 13.03	82.00 ± 12.15	Time	0.232	0.146
	12	85.67 ± 8.58	83.33 ± 13.22	T × G	0.320	0.365
PDQ-39 SI	0	27.55 ± 16.11	27.03 ± 19.32	Group	0.884	–
	7	25.24 ± 15.78	26.62 ± 18.22	Time	0.620	0.359
	12	26.04 ± 16.95	27.07 ± 19.47	T × G	0.768	0.264
Domains of PDQ-39						
Mobility	0	27.58 ± 19.78	29.17 ± 23.22	Group	0.287	–
	7	22.42 ± 19.60	32.08 ± 23.20	Time	0.676	0.030†
	12	24.25 ± 18.17	29.17 ± 22.93	T × G	0.111	0.306
Activities of	0	29.31 ± 19.50	25.56 ± 24.34	Group	0.687	–
Daily Living	7	26.81 ± 20.17	27.36 ± 23.13	Time	0.676	0.182
	12	30.42 ± 22.51	27.22 ± 24.07	T × G	0.530	0.522
Emotional	0	29.86 ± 23.85	31.67 ± 24.29	Group	0.592	–
Wellbeing	7	25.14 ± 20.19	28.47 ± 24.12	Time	0.106	0.867
	12	26.11 ± 21.24	30.00 ± 25.22	T × G	0.850	0.928
Stigma	0	24.17 ± 16.47	28.96 ± 23.81	Group	0.572	–
	7	25.83 ± 17.43	26.67 ± 23.21	Time	0.414	0.224
	12	22.92 ± 18.95	25.83 ± 24.44	T x G	0.539	0.441
Social support	0	27.22 ± 22.52	20.28 ± 19.41	Group	0.404	–
	7	24.72 ± 21.27	19.44 ± 20.57	Time	0.632	0.600
	12	24.17 ± 24.21	23.61 ± 22.11	T × G	0.289	0.060
Cognition	0	29.79 ± 21.88	25.63 ± 21.17	Group	0.616	–
	7	27.29 ± 21.49	25.63 ± 18.30	Time	0.748	0.370
	12	28.54 ± 22.72	27.08 ± 20.52	T × G	0.748	0.517
Communication	0	23.61 ± 19.34	23.06 ± 24.04	Group	0.889	–
	7	23.33 ± 19.99	23.06 ± 23.02	Time	0.857	0.836
	12	24.72 ± 19.14	23.33 ± 25.37	T × G	0.933	0.395
Bodily discomfort	0	28.89 ± 23.03	31.94 ± 21.23	Group	0.529	–
	7	26.39 ± 23.27	30.28 ± 17.84	Time	0.447	0.768
	12	27.22 ± 24.26	30.28 ± 21.27	T × G	0.942	0.927
EQ-5D Index score	0	0.72 ± 0.11	0.70 ± 0.15	Group	0.462	–
	7	0.73 ± 0.13	0.73 ± 0.12	Time	0.101	0.833
	12	0.73 ± 0.13	0.69 ± 0.16	T × G	0.417	0.561
EQ-5D VAS	0	71.70 ± 13.75	73.23 ± 16.38	Group	0.840	–
	7	68.50 ± 15.60	70.30 ± 19.10	Time	0.094	0.882
	12	73.70 ± 16.25	72.67 ± 18.88	T × G	0.694	0.420

BBT, Berg balance test; TUG, Timed Up and Go test; SE-ADL, Schwab and England Activities of Daily Living Scale; PDQ-39, 39-item Parkinson's Disease Questionnaire; PDQ-39 SI, PDQ-39 summary index; EQ-5D, five-level version of European Quality of Life Scale Five-Dimensional Questionnaire; VAS, Visual analog scale; T, Time; G, Group.
Values are presented as mean ± standard deviation.
* *P* value was calculated using repeated measures ANOVA.
† *P* value was calculated comparing differences from baseline (Δ score) between groups by independent *t*-test or Wilcoxon rank-sum test.

TABLE 8 Comparison PDQ-39 domains and summary index between groups with anxiety.

	Week	Treatment group with anxiety ($n = 17$)	Control group with anxiety ($n = 20$)	Source	*P* value*	*P* value†
PDQ-39 SI	0	31.93 ± 15.80	27.67 ± 17.61	Group	0.847	–
	7	25.17 ± 14.94	28.89 ± 17.10	Time	0.312	0.056
	12	26.37 ± 16.70	29.93 ± 18.50	T × G	0.048	0.020
Domains of PDQ-39						
Mobility	0	31.32 ± 19.12	29.63 ± 19.96	Group	0.190	–
	7	19.41 ± 17.49	35.63 ± 21.32	Time	0.365	0.001
	12	23.09 ± 14.91	31.13 ± 21.38	T × G	0.007	0.038
Activities of	0	35.29 ± 18.81	24.79 ± 21.44	Group	0.651	–
Daily Living	7	26.47 ± 19.65	29.58 ± 22.21	Time	0.695	0.013
	12	30.64 ± 21.29	29.38 ± 23.70	T × G	0.042	0.024
Emotional	0	37.75 ± 20.96	34.17 ± 19.00	Group	0.927	–
Wellbeing	7	28.92 ± 20.49	31.25 ± 21.27	Time	0.084	0.434
	12	30.15 ± 21.93	33.13 ± 23.12	T × G	0.410	0.365
Stigma	0	26.10 ± 18.25	30.31 ± 22.88	Group	0.460	–
	7	25.00 ± 17.54	29.38 ± 22.31	Time	0.465	0.981
	12	22.43 ± 17.12	27.81 ± 24.37	T × G	0.969	0.921
Social support	0	32.84 ± 24.20	19.58 ± 18.19	Group	0.320	–
	7	28.92 ± 21.67	21.25 ± 20.32	Time	0.761	0.341
	12	26.47 ± 26.72	28.33 ± 21.01	T × G	0.057	0.004
Cognition	0	35.29 ± 22.53	27.50 ± 21.69	Group	0.961	–
	7	24.63 ± 18.42	27.81 ± 19.29	Time	0.161	0.040
	12	27.21 ± 19.51	30.94 ± 21.12	T × G	0.059	0.024
Communication	0	25.49 ± 17.30	20.00 ± 21.19	Group	0.491	–
	7	24.02 ± 20.17	20.83 ± 19.21	Time	0.801	0.502
	12	25.98 ± 19.07	22.08 ± 23.77	T × G	0.898	0.774
Bodily discomfort	0	31.37 ± 23.85	35.42 ± 23.71	Group	0.184	–
	7	24.02 ± 19.07	35.42 ± 18.90	Time	0.253	0.222
	12	25.00 ± 21.45	36.67 ± 22.52	T × G	0.166	0.151

PDQ-39, 39-item Parkinson's Disease Questionnaire; PDQ-39 SI, PDQ-39 summary index; T, Time; G, Group.
P value was calculated using repeated measures ANOVA.
† *P* value was calculated comparing differences from baseline (Δ score) between groups by Mann–Whitney's *U* test.

WBC. WBC elevation was thought to be caused by a common cold, and after re-examination at a follow-up visit, it returned to normal. Aside from that, there were no adverse events with clinical abnormalities, vital signs, blood samples, or ECG.

Discussion

In comparison with the control group, the treatment group did not reveal significant changes in the MDS-UPDRS score at week 7 and other outcomes at weeks 7 and 12. In subgroup analysis of anxiety participants, a significant time x group interaction was found in the PDQ-39 domain of mobility, activities of daily living, and the PDQ-39 summary index. In the *post-hoc* analysis of PDQ-39 on patients with anxiety who responded to item 21 (anxious) of PDQ-39, significant differences between the two groups were detected in the domains of mobility and activities of daily living at week 7.

In line with these real-world clinical situations of Korea, we planned to apply acupuncture treatment in the same regimen for both the test group and the control group. Acupuncture is the most common treatment for patients with PD who visit a Korean medicine hospital for outpatient, followed by herbal medicine (22). At actual clinical sites of Korean medicine, using herbal medicine alone without acupuncture for PD is unusual unless there are extraordinary cases, such as needle phobia. There are two reasons: The first is that acupuncture is an effective and safe treatment for PD and the second is that it is covered by Korean national health insurance, so the cost burden is minimal. Therefore, acupuncture is performed in almost all patients with PD by

Korean medical doctors, but herbal medicine is administered as necessary.

The pattern identification in traditional East Asian medicine, which is a pattern of symptom manifestation that varies between individuals, is used in clinical herbal medicine to make diagnoses and prescribe medicine. And UGS was traditionally used to treat neurosis, insomnia, night crying, and irritability in children. UGS had been shown in previous studies to improve neuropsychiatric symptoms such as aggression, agitation, and anxiety in patients with PD or Alzheimer's disease (12, 13, 23–26). According to an animal study, the anti-anxiety effect of UGS might be mediated by serotonin (upregulation of 5HT1A and downregulation of 5HT2A) and glutamate release (glutamate transport by increasing express glutamate transporter mRNA) (27). Despite these scientific findings, herbal medicine may not have the same effect on everyone. Treatment that focuses on symptoms rather than diseases, such as traditional methods, might be more effective (28). In Japan, 15% of RCTs using herbal medicine included a pattern of symptom manifestation diagnosis (29). Therefore, clinical trials in combination with the traditional diagnosis are thought to be a better way to demonstrate traditional East Asian medicine's characteristics. A real-world trial was conducted in this manner, with positive results (30). Therefore, for the above reason, it is considered that the treatment group was more effective on the PDQ-39 than the control group in the *post-hoc* test of the subgroup with anxiety.

Anxiety is a common non-motor symptom in patients with PD. Concerns about PD, experiences in an "off" state, and neurotransmitter reduction are all known to cause anxiety (31). According to Hannah et al., anxiety is more important than depression in predicting quality of life of the patients with PD (32). In this study, PD patients with anxiety based on item 21 of PDQ-39 were included in the *post-hoc* analysis. Item 21, anxious, was known to have a strong relationship with the State-Trait Anxiety Inventory (STAI), the most widely used anxiety self-assessment tool (33). The STAI had also been used in several studies to assess anxiety in patients with Parkinson's disease, and it was reliable and valid (34, 35). As a result, we divided the patients into anxiety subgroups based on their responses to item 21.

In the control group with anxiety, there was no significant improvement in PDQ-39 after acupuncture + medications related to Parkinson's disease. Acupuncture's efficacy in alleviating non-motor symptoms in PD is less well known than its effect on alleviating motor symptoms. It mainly showed significant effects on depression or insomnia (6), but in PDQ-39, previous research has shown a variety of results following acupuncture treatment, making it difficult to make a definitive conclusion (36). And the negative effect may have had a role in this trial because the control group was not given placebo medicines.

This study aims to find out whether there was effectiveness and safety in the administration of UGS compared with the control group. However, there was no additional change in clinical symptoms of Parkinson's disease in the treatment group compared to the control group. Only the quality of life of participants with anxiety was significantly affected. Similar results were found in previous studies in which UGS was administered to patients with Parkinson's disease. Although UGS reduced the Neuropsychiatric Inventory (NPI) score, it did not affect the UPDRS III (motor examination) or the H–Y scale (12, 13). Furthermore, Uncaria in UGS exhibited a similar effect as Aripiprazole, an antipsychotic drug that can act as a partial dopamine agonist and cause drug-induced parkinsonism (37). When taken as above, UGS may not be able to aid in the improvement of motor function. However, UGS has been reported to increase dopamine levels with similar effects to catechol-O-methyltransferase (COMT) inhibitors, as well as protect dopamine neurons from neurotoxicity and improve drug-induced parkinsonian symptoms (10, 11, 38, 39). Moreover, UGS was also known to ameliorate dyskinetic movement caused by levodopa (40). Taking these heterogeneous findings into account, more research into the efficacy of UGS is thought to be necessary.

There are some limitations to the present study. First, in this study, the participants in the control group could not be blinded. For this reason, there may be a placebo effect in the treatment group. Second, a 6-week treatment period may not be long enough to obtain the result. Clinical trials examining the effectiveness of herbal medicines in PD had a period of almost 12 weeks or more (9). As a result, the 6-week period may have been insufficient to see the intact effect of UGS. However, even in a short period, the administration of UGS showed significant effectiveness in the anxiety group. Finally, there were mixed cases where "on" and "off" were not the same at the time of measurement. In this trial, "on" and "off" states were analyzed together. However, the "off"-state participant was few, and it is estimated that their impact will not be significant.

Despite the limitations mentioned above, this research had some advantages. This study was a pilot clinical trial to verify the efficacy of herbal medicine UGS based on recent experimental results. The study found that the clinical effectiveness of UGS on QoL examined with PDQ-39 was estimated to be significantly larger in patients with PD and anxiety. The findings of this study can be used as a base for large-scale clinical trials on the efficacy of UGS in the treatment of PD in future.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Kyung Hee University Korean Medicine Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CJ, K-HC, and SK conceived the objectives of the study. CJ acquired the data. CJ, B-KK, H-JP, and SK analyzed the data and performed the statistical analysis, which was discussed with W-SJ and T-HK. CJ, K-HC, and SK wrote the first draft of the manuscript. CJ, W-SJ, H-GL, S-YC, S-KM, C-NK, H-JP, and SK co-drafted the final version. K-HC and SK supervised the study. All authors critically revised the manuscript and have approved the final manuscript.

Funding

This work is supported by the grant from the Traditional Korean Medicine R&D Program and funded by the Ministry of Health and Welfare through the Korea Health Industry Development Institute (KHIDI, HB16C0051) and the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (No. 2022M3A9B6017813).

Acknowledgments

This paper is based on CJ doctoral dissertation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. *Nat Rev Dis Primers.* (2017) 3:1–21. doi: 10.1038/nrdp.2017.13

2. Dorsey E, Sherer T, Okun MS, Bloem BR. The emerging evidence of the Parkinson pandemic. *J Parkinson's Dis.* (2018) 8:S3–8. doi: 10.3233/JPD-181474

3. Kalia LV, Lang AE. Parkinson's disease. *Lancet.* (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3

4. Bega D, Zadikoff C. Complementary & alternative management of Parkinson's disease: an evidence-based review of eastern influenced practices. *J Movement Disorders.* (2014) 7:57. doi: 10.14802/jmd.14009

5. Lee S-H, Lim S. Clinical effectiveness of acupuncture on Parkinson disease: a PRISMA-compliant systematic review and meta-analysis. *Medicine.* (2017) 96:e5836. doi: 10.1097/MD.0000000000005836

6. Huang J, Qin X, Cai X, Huang Y. Effectiveness of acupuncture in the treatment of Parkinson's disease: an overview of systematic reviews. *Front Neurol.* (2020) 11:917. doi: 10.3389/fneur.2020.00917

7. Ko JH, Lee H, Kim S-N, Park H-J. Does acupuncture protect dopamine neurons in Parkinson's disease rodent model?: a systematic review and meta-analysis. *Front Aging Neurosci.* (2019) 11:102. doi: 10.3389/fnagi.2019.00102

8. Shan C-S, Zhang H-F, Xu Q-Q, Shi Y-H, Wang Y, Li Y, et al. Herbal medicine formulas for Parkinson's disease: a systematic review and meta-analysis of randomized double-blind placebo-controlled clinical trials. *Front Aging Neurosci.* (2018) 10:349. doi: 10.3389/fnagi.2018.00349

9. Zhang G, Xiong N, Zhang Z, Liu L, Huang J, Yang J, et al. Effectiveness of traditional Chinese medicine as an adjunct therapy for Parkinson's disease: a systematic review and meta-analysis. *PLoS ONE.* (2015) 10:e0118498. doi: 10.1371/journal.pone.0118498

10. Doo A-R, Kim S-N, Park J-Y, Cho KH, Hong J, Eun-Kyung K, et al. Neuroprotective effects of an herbal medicine, Yi-Gan San on MPP+/MPTP-induced cytotoxicity *in vitro* and *in vivo*. *J Ethnopharmacol.* (2010) 131:433–42. doi: 10.1016/j.jep.2010.07.008

11. Ishida Y, Ebihara K, Tabuchi M, Imamura S, Sekiguchi K, Mizoguchi K, et al. Yokukansan, a traditional Japanese medicine, enhances the L-DOPA-induced rotational response in 6-hydroxydopamine-lesioned rats: possible inhibition of COMT. *Biol Pharmaceut Bull.* (2016) 39:104–13. doi: 10.1248/bpb.b15-00691

12. Hatano T, Hattori N, Kawanabe T, Terayama Y, Suzuki N, Iwasaki Y, et al. An exploratory study of the efficacy and safety of yokukansan for neuropsychiatric symptoms in patients with Parkinson's disease. *J Neural Transm.* (2014) 121:275–81. doi: 10.1007/s00702-013-1105-y

13. Kawanabe T, Yoritaka A, Shimura H, Oizumi H, Tanaka S, Hattori N. Successful treatment with Yokukansan for behavioral and psychological symptoms of Parkinsonian dementia. *Prog Neuro-Psychopharmacol Biol Psychiatry.* (2010) 34:284–7. doi: 10.1016/j.pnpbp.2009.11.019

14. MacPherson H, Altman DG, Hammerschlag R, Youping L, Taixiang W, White A, et al. Revised standards for reporting interventions in clinical trials of acupuncture (STRICTA): extending the CONSORT statement. *J Alternat Complement Med.* (2010) 16:ST-1-ST-14. doi: 10.1089/acm.2010.1610

15. Goetz CG, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stebbins GT, et al. Movement disorder society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): process, format, and clinimetric testing plan. *Movement Disorders.* (2007) 22:41–7. doi: 10.1002/mds.21198

16. Jenkinson C, Fitzpatrick R, Peto V, Greenhall R, Hyman N. The Parkinson' disease questionnaire (PDQ-39): development and validation of a Parkinson's disease summary index score. *Age Ageing.* (1997) 26:353–7. doi: 10.1093/ageing/26.5.353

17. Kim S-H, Ahn J, Ock M, Shin S, Park J, Luo N, et al. The EQ-5D-5L valuation study in Korea. *Qual Life Res.* (2016) 25:1845–52. doi: 10.1007/s11136-015-1205-2

18. Pan W, Kwak S, Liu Y, Sun Y, Fang Z, Qin B, et al. Traditional chinese medicine improves activities of daily living in Parkinson's disease. *Parkinson's Dis.* (2011) 2011:789506. doi: 10.4061/2011/789506

19. Shulman LM, Gruber-Baldini AL, Anderson KE, Fishman PS, Reich SG, Weiner WJ. The clinically important difference on the unified Parkinson's disease rating scale. *Arch Neurol.* (2010) 67:64–70. doi: 10.1001/archneurol.2009.295

20. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Stat Methods Med Res.* (2016) 25:1057–73. doi: 10.1177/0962280215588241

21. Shi J, Tian J, Li T, Qin B, Fan D, Ni J, et al. Efficacy and safety of SQJZ herbal mixtures on nonmotor symptoms in Parkinson disease patients: protocol for a randomized, double-blind, placebo-controlled trial. *Medicine*. (2017) 96:e8824. doi: 10.1097/MD.0000000000008824

22. Yang JY, Jin C, Lee J, Lee H-g, Cho S-Y, Park S-U, et al. Patients with Parkinson disease in a traditional korean medicine hospital: a five-year audit. *Evidence Based Complement Alternat Med.* (2021) 2021:6842863. doi: 10.1155/2021/6842863

23. Mizukami K, Asada T, Kinoshita T, Tanaka K, Sonohara K, Nakai R, et al. A randomized cross-over study of a traditional Japanese medicine (kampo), yokukansan, in the treatment of the behavioural and psychological symptoms of dementia. *Int J Neuropsychopharmacol.* (2009) 12:191–9. doi: 10.1017/S146114570800970X

24. Hayashi Y, Ishida Y, Inoue T, Udagawa M, Takeuchi K, Yoshimuta H, et al. Treatment of behavioral and psychological symptoms of Alzheimer-type dementia with Yokukansan in clinical practice. *Prog Neuro-Psychopharmacol Biol Psychiatry.* (2010) 34:541–5. doi: 10.1016/j.pnpbp.2010.02.016

25. Iwasaki K, Kosaka K, Mori H, Okitsu R, Furukawa K, Manabe Y, et al. Improvement in delusions and hallucinations in patients with dementia with Lewy bodies upon administration of yokukansan, a traditional Japanese medicine. *Psychogeriatrics.* (2012) 12:235–41. doi: 10.1111/j.1479-8301.2012.00413.x

26. Nagata K, Yokoyama E, Yamazaki T, Takano D, Maeda T, Takahashi S, et al. Effects of yokukansan on behavioral and psychological symptoms of vascular dementia: an open-label trial. *Phytomedicine.* (2012) 19:524–8. doi: 10.1016/j.phymed.2012.02.008

27. Ikarashi Y, Mizoguchi K. Neuropharmacological efficacy of the traditional Japanese Kampo medicine yokukansan and its active ingredients. *Pharmacol Ther.* (2016) 166:84–95. doi: 10.1016/j.pharmthera.2016.06.018

28. Lu A-P, Jia H-W, Xiao C, Lu Q-P. Theory of traditional Chinese medicine and therapeutic method of diseases. *World J Gastroenterol. WJG.* (2004) 10:1854. doi: 10.3748/wjg.v10.i13.1854

29. Motoo Y, Arai I, Tsutani K. Use of Kampo diagnosis in randomized controlled trials of Kampo products in Japan: a systematic review. *PLoS ONE.* (2014) 9:e104422. doi: 10.1371/journal.pone.0104422

30. Liang B, Zou F-H, Fu L, Liao H-L. Chinese herbal medicine Dingji Fumai decoction for ventricular premature contraction: a real-world trial. *BioMed Res Int.* (2020). doi: 10.1155/2020/5358467

31. Chen JJ, Marsh L. Anxiety in Parkinson's disease: identification and management. *Ther Adv Neurol Disord.* (2014) 7:52–9. doi: 10.1177/1756285613495723

32. Hanna KK, Cronin-Golomb A. Impact of anxiety on quality of life in Parkinson's disease. *Parkinson's Disease.* (2012) 2012:640707. doi: 10.1155/2012/640707

33. Jones JD, Hass C, Mangal P, Lafo J, Okun MS, Bowers D. The cognition and emotional well-being indices of the Parkinson's disease questionnaire-39: what do they really measure? *Parkinsonism Relat Disord.* (2014) 20:1236–41. doi: 10.1016/j.parkreldis.2014.09.014

34. Matheson SF, Byrne GJ, Dissanayaka NN, Pachana NA, Mellick GD, O'Sullivan JD, et al. Validity and reliability of the geriatric anxiety inventory in Parkinson's disease. *Australas J Ageing.* (2012) 31:13–6. doi: 10.1111/j.1741-6612.2010.00487.x

35. Mondolo F, Jahanshahi M, Granà A, Biasutti E, Cacciatori E, Di Benedetto P. Evaluation of anxiety in Parkinson's disease with some commonly used rating scales. *Neurol Sci.* (2007) 28:270–5. doi: 10.1007/s10072-007-0834-9

36. Wen X, Li K, Wen H, Wang Q, Wu Z, Yao X, et al. Acupuncture-related therapies for Parkinson's disease: a meta-analysis and qualitative review. *Front Aging Neurosci.* (2021) 13:676827. doi: 10.3389/fnagi.2021.676827

37. Ueda T, Ugawa S, Ishida Y, Shimada S. Geissoschizine methyl ether has third-generation antipsychotic-like actions at the dopamine and serotonin receptors. *Eur J Pharmacol.* (2011) 671:79–86. doi: 10.1016/j.ejphar.2011.09.007

38. Eo H, Huh E, Sim Y, Oh MS. Ukgansan protects dopaminergic neurons from 6-hydroxydopamine neurotoxicity *via* activation of the nuclear factor (erythroid-derived 2)-like 2 factor signaling pathway. *Neurochem Int.* (2019) 122:208–15. doi: 10.1016/j.neuint.2018.11.021

39. Shim Y-H, Park J-Y, Choi W-W, Min I-K, Park S-U, Jung W-S, et al. Herbal medicine treatment for drug-induced parkinsonism. *J Alternative Complement Med.* (2015) 21:273–80. doi: 10.1089/acm.2014.0124

40. Huh E, Choi JG, Sim Y, Oh MS. An integrative approach to treat parkinson's disease: Ukgansan complements L-Dopa by ameliorating dopaminergic neuronal damage and L-dopa-induced dyskinesia in mice. *Front Aging Neurosci.* (2019) 10:431. doi: 10.3389/fnagi.2018.00431

frontiers | Frontiers in Neurology

TYPE Brief Research Report
PUBLISHED 23 November 2022
DOI 10.3389/fneur.2022.1052989

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Raffaele Dubbioso,
Federico II University Hospital, Italy
Josko Soda,
University of Split, Croatia

*CORRESPONDENCE
Massimo Marano
m.marano@policlinicocampus.it

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 24 September 2022
ACCEPTED 03 November 2022
PUBLISHED 23 November 2022

CITATION
Cruciani A, Lanzone J, Musumeci G, Di Lazzaro V and Marano M (2022) Focal vibrations enhance somatosensory facilitation in healthy subjects: A pilot study on Equistasi® and high-frequency oscillations. *Front. Neurol.* 13:1052989. doi: 10.3389/fneur.2022.1052989

Focal vibrations enhance somatosensory facilitation in healthy subjects: A pilot study on Equistasi® and high-frequency oscillations

Alessandro Cruciani[1,2], Jacopo Lanzone[3], Gabriella Musumeci[1,2], Vincenzo Di Lazzaro[1,2] and Massimo Marano[1,2]*

[1]Research Unit of Neurology, Neurophysiology and Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy, [2]Operative Research Unit of Neurology, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy, [3]Istituti Clinici Scientifici Maugeri IRCCS, Neurorehabilitation Unit of Milan Institute, Milan, Italy

Background: Equistasi® is a vibrotactile device composed of nanotechnology fibers that converts temperature change into mechanical energy by self-producing a focal vibration. It is used in non-pharmacological rehabilitation in patients with movement disorders and multiple sclerosis sequelae. Nonetheless, the mechanism underlying such an improvement in motor functions is still poorly understood.

Objectives: We designed a small uncontrolled pilot trial to explore the effect of Equistasi® on the somatosensory pathway through the analysis of high-frequency oscillations (HFOs).

Methods: For all the included subjects, we recorded somatosensory-evoked potentials (SEPs) at the baseline (T0) and at 60 min after the application of Equistasi® (T1) on the seventh cervical vertebra level and at the forearm over each flexor carpi radialis, bilaterally. Then, we extracted the HFOs from the N20 signal and compared the HFO duration and area under the curve pre- and post-Equistasi® application.

Results: In a head-to-head comparison of T0 to T1 data, there was a statistically significant reduction in the total HFO area ($p < 0.01$), which was prominent for the late component ($p = 0.025$). No statistical differences have been found between T0 and T1 HFO duration ($p > 0.05$). We further evaluated the N20 amplitude from the onset to the N20 peak to avoid possible interpretational bias. No statistical differences have been found between T0 and T1 ($p = 0.437$).

Conclusion: Our clinical hypothesis, supported by preliminary data, is that vibrotactile afference delivered by the device could work by interfering with the somatosensory processing, rather than by peripheral effects.

KEYWORDS

focal vibration, high-frequency oscillation, movement disorders, non-pharmacological rehabilitation, Equistasi®, somatosensory processing

Introduction

Equistasi® is a vibrotactile device composed of nanotechnology fibers. When this small tool is worn on the body, it converts the temperature change, due to the contact with the skin, into mechanical energy by self-producing a focal vibration (1). Applying an Equistasi® device over a muscle tendon supposedly modulates the Golgi mechanoreceptor activity, which is recognized as the proprioceptive system gate. The application of Equistasi® in clinical practice is supported by randomized trials conducted on movement disorders, such as Parkinson's disease or multiple sclerosis sequelae (1, 2). Its effect is mainly observed in balance, gait, and overall motor function and is likely mediated by sensory feedback modulation of the proprioceptive system. Nevertheless, the mechanism underlying such an improvement in motor functions is still poorly understood. Somatosensory pathways and sensory motor integration have a pivotal role in modulating the motor output, as also suggested by the application of sensory cueing in movement disorders, such as Parkinson's disease or dystonia (i.e., sensory tricks) (3). Nevertheless, the complete mechanisms of action are still not fully understood, and studies that aim at investigating the neurophysiological basis of proprioceptive devices on somatosensory pathways are lacking. High-frequency oscillations (HFOs) are a well-established neurophysiological marker to evaluate somatosensory processing (4, 5). HFOs are fast physiological oscillations that underpin somatosensory-evoked potential. These waves are obtained by applying digital high-pass filtering on low-frequency median SEP to divide the signal from the original N20 response (6). Such oscillations are subdivided into an early and a late component based on the peak of the N20. Early HFOs measure thalamocortical input, while late HFOs reflect the activity of intracortical GABAergic interneurons located in the somatosensory cortex (6). Hence, we designed a small uncontrolled pilot trial to explore the effect of Equistasi® on somatosensory processing through the evaluation of high-frequency oscillations (HFOs), which change pre- and post-Equistasi® applications.

Methods

Participants

A total of 10 right-handed healthy volunteers (four women, six men; median age 21.5 ± 2.9 years), were consecutively enrolled by the school of medicine of our university. The handedness of the participants was tested by using the Edinburgh Handedness Inventory (7). The study was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee. The participants signed a regular informed consent.

Study design

For all the enrolled subjects, we recorded somatosensory-evoked potentials (SEPs) at the baseline (T0) and at 60 min after the application of Equistasi® (T1).

The device was applied over the skin at the 7th cervical vertebra level as suggested by the manufacturer, and at the forearm over each flexor carpi radialis (i.e., a median nerve innervated muscle), bilaterally.

Neurophysiological assessment

The median nerve SEP was evoked by conventional electrical stimulation at the wrist of the dominant hand using a high-voltage stimulator (DS7A, Digitimer Ltd, UK). We used bar electrodes; the anode was placed on the wrist crease, while the cathode was placed proximally. In total, 1,200 pulses of 200-μs duration were delivered at a frequency of 1.9 Hz. We used the lower intensity capable of generating a slight thumb twitch. Ag/AgCl surface electrodes were placed at left CP3 (active electrode) and Fz (reference electrode) locations of the international 10/20 system. The 1,200 sweeps were averaged, bandpass-filtered (0.5–2,000 Hz), and digitized at a sample rate of 5 kHz using a portable amplifier (BrainVision Recorder, BrainAmp MR plus, Brain Products GmBH, Germany, version:1.10).

Data analysis

Data were analyzed by *ad hoc* MATLAB (MathWorks, Inc., Massachusetts, USA, version: R2021b) script. A digital 400–800 Hz bandpass Butterworth filter was applied to extract HFOs. The area and duration were calculated from the rectified data from the point at which upward deflection was more than 50% of the background noise to the point where deflection was <50% (8). The N20 features and the HFO area and duration were then compared across conditions.

Statistical analysis

The statistical analysis was performed using SPSS (IBM Corp., Armonk, New York, USA, version 25). All the results are expressed as mean ± standard deviation. Data were compared using the Wilcoxon signed-rank test or the *t*-test for paired data according to their distribution (Shapiro–Wilk test) and were corrected for multiple comparisons according to the Benjamini–Hochberg procedure, with a false discovery rate of 0.05. The experiment was well tolerated, and no dropouts were reported.

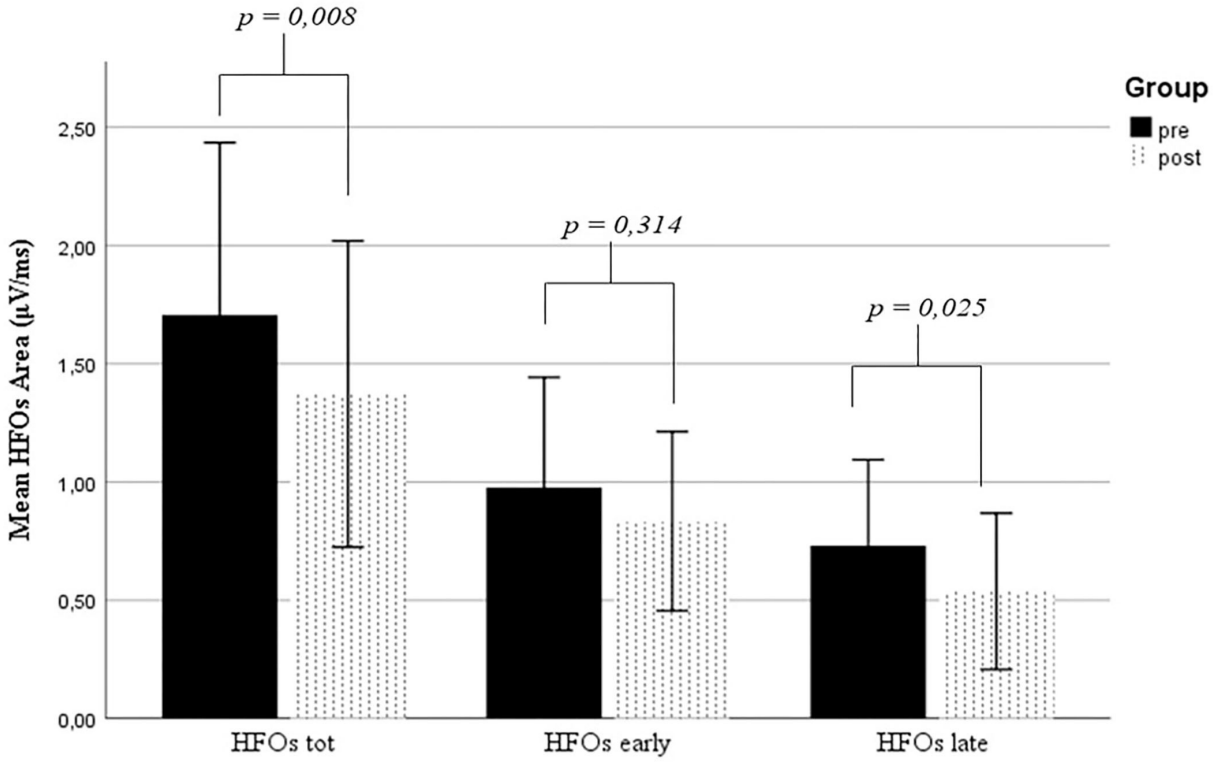


FIGURE 1
Comparison of HFO values between T0 and T1. HFOs, high-frequency oscillations.

Results

The N20 latency was 19.6 ms ± 1.6 at the baseline (T0) and remained stable at T1. At the baseline (T0), the HFO presented a total AUC of 1.705 μV/ms (SD = ±1.153), subdivided into early (0.975 μV/ms ± 0.737), and late (0.730 μV/ms ± 0.574) HFOs. Similar findings were observed at T1 (total AUC = 1.372 μV/ms ± 1.023; early AUC = 0.834 μV/ms ± 0.598; late AUC = 0.537 μV/ms ± 0.522). In a head-to-head comparison of T0 to T1 data, there was a statistically significant global reduction in the total HFO area ($p < 0.01$), which was prominent for the late component ($p = 0.025$) (Figure 1). No statistical differences have been found between T0 and T1 HFO duration ($p > 0.05$). The latter showed a total value of 8.8 ms ± 1.9 and 8.3 ms ± 2 at T0 and T1, respectively. The early HFO duration at T0 was 4.8 ms ± 1.3 and 4.5 ms ± 1.5 at T1, while the late HFO duration was 2.8 ms ± 2.2 at T0 and 2.5 ms ± 2.1 at T1. We further evaluated the N20 amplitude from the onset to the N20 peak to avoid possible interpretational bias. At T0, the onset-to-peak N20 amplitude was 1.8 μV ± 0.8, while at T1, the amplitude was 1.9 μV ± 0.9. No statistical differences have been found comparing the onset-to-peak N20 amplitude between T0 and T1 ($p = 0.437$). The main results are summarized in Table 1.

Discussion

HFO is a powerful neurophysiological tool to assess the integrity of the somatosensory pathway (5). Since the cortical (i.e., late) part derives from intracortical GABAergic interneurons, and the early part reflects the thalamocortical section, HFO is widely used in the study of pathophysiological mechanisms in which alteration of the somatosensory system is believed to be a pathophysiological mechanism (8–13). One of the first applications of HFO in the clinic practice was in the field of epilepsy (9, 10). In conditions such as juvenile myoclonus epilepsy (JME) or familial adult myoclonic epilepsy type 2 (FAME2), the alteration of HFOs, especially in the cortical parts, help identify the contribution of the somatosensory system hyperexcitability in the epileptic susceptibility of the patients (9, 10). Furthermore, studies conducted on patients with Parkinson's disease (11, 13) and cervical dystonia found a modification in the late HFO component. Specifically, patients with PD showed enhanced HFOs than healthy controls (11, 12). One possible explanation for the enhanced HFOs is based on the interaction between the basal ganglia and the somatosensory system in patients with PD. Indeed, in patients with PD, the GABAergic impairment of neurons located in the external part of the globus pallidus could lead to disinhibition of GABAergic

TABLE 1 HFOs and N20 values expressed as means ± standard deviation.

	T0	T1	*P*-value
Total HFOs AUC (μV/ms)	1.705 ± 1.153	1.372 ± 1.023	0.008
Early HFOs AUC (μV/ms)	0.975 ± 0.737	0.834 ± 0.598	0.314
Late HFOs AUC (μV/ms)	0.730 ± 0.574	0.537 ± 0.522	0.025
Total HFOs duration (ms)	8.8 ± 1.9	8.3 ± 2	0.075
Early HFOs duration (ms)	4.8 ± 1.3	4.5 ± 1.5	0.089
Late HFOs duration (ms)	2.8 ± 2.2	2.5 ± 2.1	0.352
Onset-to-peak N20 Amplitude (μV)	1.8 ± 0.8	1.9 ± 0.9	0.437

HFOs, high-frequency oscillations; AUC, area under the curve.

interneurons in the thalamic reticular nucleus. Subsequently, the thalamocortical projection is diminished, causing a reduction in the activity of interneuron layer IV in the sensory cortex (11, 12), which are believed to be the generators of late HFOs (14). Interestingly, contrasting results have been found for patients with dystonia, with a decrease in GABAergic activity leading to smaller HFOs (12). Hence, in patients with dystonia, the device could interact with the aforementioned cortical pathways, restoring the physiological equilibrium. In our study, we found significant effects of Equistasi® application on the cortical part of HFOs (AUC reduction of ∼25%), similarly to that observed in rTMS experiments (15). One hypothesis is that the vibrotactile stimulation delivered by Equistasi® generates a sensory signal that enters the central nervous system from the Golgi mechanoreceptors. Thus, the vibrotactile signal passes through the dorsal column and ultimately terminates in the ventral posterolateral (VPL) nucleus of the thalamus. Here, it takes synapses with GABAergic neurons (16).

Conclusion

Indeed, our clinical hypothesis supported by preliminary data is that vibrotactile afference delivered by the device could work by interfering with somatosensory processing, rather than by peripheral effects. The interpretation of our results is limited by the small population and uncontrolled study design and deserves further experiments. Another limitation is the median age of the population, which is not completely representative of the typical age of patients with neurological diseases, such as Parkinson's disease or dystonia. This difference makes our study findings difficult to be generalized to neurological disorders. However, if proven true, the reduction in the late HFO part induced by Equistasi® might be hypothetically of help in restoring the equilibrium between somatosensory and motor pathways, which have been thoroughly investigated in Parkinson's disease, dystonia, and related disorders. The potential application of this device in patients with dystonia is particularly interesting since there is an acceptability issue in current therapies—that is, botulinum toxin. Indeed, Equistasi® could represent a non-invasive valid option in dystonia treatment. Moreover, our study considers one neurophysiological tool, so future studies should consider other parameters, such as short intracortical inhibition (SICI) and short afferent inhibition (SAI). Hence, future controlled trials with a more conspicuous sample size evaluating the effects of the device in healthy subjects and in patients with dystonia and PD are needed to confirm this preliminary data.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Università Campus Bio-Medico di Roma. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MM: conceptualization. AC, JL, GM, VD, and MM: methodology. VD and MM: supervision. AC and MM: writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Equistasi®. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, and the decision to submit it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher,

the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Volpe D, Giantin MG, Fasano A. A wearable proprioceptive stabilizer (Equistasi®) for rehabilitation of postural instability in Parkinson's disease: a phase II randomized double-blind, double-dummy, controlled study. *PLoS ONE.* (2014) 9:e112065. doi: 10.1371/journal.pone.0112065

2. Peppe A, Paravati S, Baldassarre MG, Bakdounes L, Spolaor F, Guiotto A, et al. Proprioceptive focal stimulation (Equistasi®) may improve the quality of gait in middle-moderate Parkinson's disease patients. Double-blind, double-dummy, randomized, crossover, Italian multicentric study. *Front Neurol.* (2019) 10:998. doi: 10.3389/fneur.2019.00998

3. Delgado-Alvarado M, Marano M, Santurtún A, Urtiaga-Gallano A, Tordesillas-Gutierrez D, Infante J. Nonpharmacological, nonsurgical treatments for freezing of gait in Parkinson's disease: a systematic review. *Mov Disord.* (2020) 35:204–14. doi: 10.1002/mds.27913

4. Motolese F, Rossi M, Capone F, Cruciani A, Musumeci G, Manzo M, et al. High-frequency oscillations-based precise temporal resolution of short latency afferent inhibition in the human brain. *Clin Neurophysiol.* (in press). doi: 10.1016/j.clinph.2022.09.006

5. Ozaki I, Hashimoto I. Exploring the physiology and function of high-frequency oscillations (HFOs) from the somatosensory cortex. *Clin Neurophysiol.* (2011) 122:1908–23. doi: 10.1016/j.clinph.2011.05.023

6. Lanzone J, Boscarino M, Ricci L, Insola A, Tombini M, Di Lazzaro V, et al. The effects of antiepileptic drugs on high-frequency oscillations in somatosensory evoked potentials. *Clin Neurophysiol.* (2020) 131:1917–24. doi: 10.1016/j.clinph.2020.05.022

7. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia.* (1971) 9:97–113.

8. Capone F, Motolese F, Rossi M, Musumeci G, Insola A, Di Lazzaro V. Thalamo-cortical dysfunction contributes to fatigability in multiple sclerosis patients: a neurophysiological study. *Mult Scler Relat Disord.* (2020) 39:101897. doi: 10.1016/j.msard.2019.101897

9. Dubbioso R, Striano P, Tomasevic L, Bilo L, Esposito M, Manganelli F, et al. Abnormal sensorimotor cortex and thalamo-cortical networks in familial adult myoclonic epilepsy type 2: pathophysiology and diagnostic implications. *Brain Commun.* (2022) 4:fcac037. doi: 10.1093/braincomms/fcac037

10. Assenza G, Lanzone J, Dubbioso R, Coppola A, Boscarino M, Ricci L, et al. Thalamic and cortical hyperexcitability in juvenile myoclonic epilepsy. *Clin Neurophysiol.* (2020) 131:2041–6. doi: 10.1016/j.clinph.2020.04.164

11. Mochizuki H, Ugawa Y, Machii K, Terao Y, Hanajima R, Furubayashi T, et al. Somatosensory evoked high-frequency oscillation in Parkinson's disease and myoclonus epilepsy. *Clin Neurophysiol.* (1999) 110:185–91.

12. Inoue K, Hashimoto I, Shirai T, Kawakami H, Miyachi T, Mimori Y, et al. Disinhibition of the somatosensory cortex in cervical dystonia: decreased amplitudes of high-frequency oscillations. *Clin Neurophysiol.* (2004) 115:1624–30. doi: 10.1016/j.clinph.2004.02.006

13. Inoue K, Hashimoto I, Nakamura S. High-frequency oscillations in human posterior tibial somatosensory evoked potentials are enhanced in patients with Parkinson's disease and multiple system atrophy. *Neurosci Lett.* (2001) 297:89–92. doi: 10.1016/S0304-3940(00)01678-5

14. Swadlow HA, Beloozerova IN, Sirota MG. Sharp, local synchrony among putative feed-forward inhibitory interneurons of rabbit somatosensory cortex. *J Neurophysiol.* (1998) 79:567–82.

15. Murakami T, Sakuma K, Nomura T, Nakashima K, Hashimoto I. High-frequency oscillations change in parallel with short-interval intracortical inhibition after theta burst magnetic stimulation. *Clin Neurophysiol.* (2008) 119:301–8. doi: 10.1016/j.clinph.2007.10.012

16. Ralston HJ, Ralston DD. Medial lemniscal and spinal projections to the macaque thalamus: an electron microscopic study of differing GABAergic circuitry serving thalamic somatosensory mechanisms. *J Neurosci.* (1994) 14:24 85–502.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 04 January 2023
DOI 10.3389/fneur.2022.1017005

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Gema P. Sáez-Suanes,
Nebrija University, Spain
Ala Aljubour,
Ministry of Health, Saudi Arabia

*CORRESPONDENCE
Moonju Kim
moonju-kim@hanmail.net

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 11 August 2022
ACCEPTED 21 November 2022
PUBLISHED 04 January 2023

CITATION
Lee B, Park S, Kwon HJ, Hwang GS and Kim M (2023) Integrative treatment program for the treatment of children with autism spectrum disorder: A prospective observational case series. *Front. Neurol.* 13:1017005. doi: 10.3389/fneur.2022.1017005

Integrative treatment program for the treatment of children with autism spectrum disorder: A prospective observational case series

Boram Lee[1], Serin Park[2], Hyun Jeong Kwon[2], Gwi Seo Hwang[3] and Moonju Kim[4]*

[1]KM Science Research Division, Korea Institute of Oriental Medicine, Daejeon, South Korea, [2]Floortime Center Korea, Seoul, South Korea, [3]College of Korean Medicine, Gachon University, Seongnam, South Korea, [4]I-Tomato Korean Medicine Clinic, Seoul, South Korea

Background: In a situation where conventional treatments for autism spectrum disorder (ASD) are labor-intensive and there are concerns about the side effects of conventional medications, a 6-month integrative treatment program, including herbal medicine (HM), Floortime, and sensory enrichment therapy (SET) has been used on children with ASD in Korean medicine clinical settings.

Methods: We observed the treatment responses of 18 children with ASD (66.7% male, mean age 3.9 ± 0.9 years) to the integrative treatment program as part of a prospective, single-center, observational case series. Individualized HMs were administered according to the patient's symptoms, and parents were instructed to perform Floortime and SET with their children at home for 2 h and 20 min a day, 5 days a week, respectively. The Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) were used to evaluate the core symptoms of ASD. A linear mixed model for repeated measures was used for analyzing the effect of the program over time, and logistic regression used to explore the predictors of treatment response.

Results: The CARS and ABC scores were significantly improved from 34.58 ± 6.27 and 69.28 ± 15.73 at baseline to 28.56 ± 6.05 and 39.67 ± 20.36 after 6 months ($p < 0.0001$, respectively). No serious adverse events (AEs) were reported, and compliance with HM, Floortime, and SET was high at >90%.

Conclusion: This 6-month integrative treatment program appears to be a potentially effective, safe, and feasible option for children with ASD. Low baseline CARS scores may be predictors of higher treatment response.

KEYWORDS

integrative medicine, autism spectrum disorder, herbal medicine, Floortime, sensory enrichment therapy, case series

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by disturbances in social communication and interaction, as well as limited and repetitive behaviors, interests, and activities (1). The onset of ASD symptoms is usually before 2 years of age, and it was estimated that an average of 1 in 44 (2.3%) 8-year-old children had ASD in 2018 (2). Since this disorder occurs from the beginning of the life cycle and the illness period is long in many cases, the burden of indirect costs such as loss of income and productivity, as well as direct costs such as medical expenses, is large (3).

The conventional treatments for ASD include behavioral therapy and medication. However, because behavioral therapies involve the use of extensive and costly resources, their access may be limited (4). In addition, adverse events (AEs) such as drowsiness, somnolence, anxiety, hypersalivation, and elevation of prolactin levels have been reported with risperidone (5). Therefore, approximately 88% of the geographically diverse sample of children with ASD in the United States of America (USA) have used complementary and integrative medicine (CIM) (6), either to treat various symptoms, including hyperactivity and inattention (7), or because of concerns about the side effects of conventional treatments (8).

Herbal medicine (HM), one of the components of CIM, has been used for the treatment of ASD for many years in clinical settings, resulting in the improvement of ASD, as reported in various studies (9). In addition, the effectiveness of parents' direct involvement in the treatment of patients with ASD has been recognized through several studies (10, 11), such involvement has the advantage of building a positive emotional relationship between parents and patients in their home environment (12). Among direct parental involvement interventions, developmental, individual difference, relationship-based (DIR)/Floortime™ therapy (Floortime), and sensory enrichment therapy (SET) have also been used for the treatment of ASD in the clinical setting (13), and the effectiveness of individual treatments has been reported in various studies (14, 15). Floortime was developed by Greenspan, an American pediatrician, and is a developmental, multi-disciplinary, play-based framework that emphasizes building connections with others and underlines the meaningful, spontaneous use of language and communication (16). This therapy involves asking parents to "get down on the floor" and play with their child for a specified period, following the child's lead during play sessions and extending what the child does to elicit as many reciprocal interactions as possible. Sensory enrichment therapy is a form of therapy that uses two or more senses simultaneously, which can significantly reduce discomfort experienced by the autistic brain (17). Sensory enrichment therapy increased the size and weight of rats' brains, enriching more brain cells, more connections, and a stronger auxiliary system to support enhanced brain activity (18). Sensory enrichment therapy provides step-by-step training methods tailored to each patient's treatment goals by a qualified therapist, covering sensory processing, learning, memory, eating habits, sleeping habits, communication, sociality, fine motor skills, self-awareness, emotions and behaviors, attention, and anxiety. Based on an algorithm through periodic questionnaires and goals, a kit consisting of 3–4 worksheets for sensory development optimized for each patient is provided once a week. The kit has the advantage of convenient accessibility because it can be carried out with items used at home daily and without special tools. The SET kit activates a new brain area by simultaneously stimulating two or more senses (e.g., olfactory, and visual). After parents are educated about various sensations and movements by a professional therapist, they directly use the kit to treat their children at home (17).

In Korean medicine clinical settings, an integrative treatment program, including HM, Floortime, and SET, has been used for many years. However, to the best of our knowledge, there is no report on this integrative treatment program for ASD, which reflects real-world practice. Therefore, we report the results of our integrative treatment program in children with ASD through a prospective observational study, identify factors related to the treatment response, and provide evidence for a more rigorous study design.

Methods

Study design

This is a prospective, single-center, pragmatic observational case series. This study was approved by the Institutional Review Board of Gachon University (1044396-201812-HR-223-01), written informed consent was obtained from all patients before enrolling in this study, and their clinical records and information were anonymized and de-identified before analysis, by assigning an individual identification code to each patient.

Patients and eligibility

Eligible patients were recruited in the outpatient setting of the I-Tomato Korean Medicine Clinic (Seoul, Republic of Korea) from March 2019 to July 2021. The inclusion criteria were (1) children aged 2–5 years, and (2) children diagnosed with ASD through the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), and the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The exclusion criteria were as follows: (1) patients with neurological abnormalities such as cerebral palsy and Down syndrome, (2) patients diagnosed with other psychiatric diseases such as

TABLE 1 Details of herbal medicine used.

Herbal medicine	Composition and doses (per day)	No. of patients (%)
Shihogyeji-tang	Bupleuri Radix 6 g, Scutellariae Radix 3 g, Pinelliae Tuber 3 g, Ginseng Radix 3 g, Cinnamomi Ramulus 4 g, Paeoniae Radix 6 g, Zingiberis Rhizoma Recens 4 g, Zizyphi Fructus 4 g, Glycyrrhizae Radix et Rhizoma 2 g	17 (94.4)
Galgeun-tang	Cinnamomi Ramulus 4 g, Paeoniae Radix 4 g, Zingiberis Rhizoma Recens 4 g, Zizyphi Fructus 4 g, Glycyrrhizae Radix et Rhizoma 2 g, Puerariae Radix 5 g, Ephedrae Herba 1 g	17 (94.4)
Baekho-tang	Anemarrhenae Rhizoma 5 g, Oryzae Semen 8 g, Gypsum Fibrosum 15 g, Glycyrrhizae Radix et Rhizoma 2 g	6 (33.3)
Daeseunggi-tang	Rhei Radix et Rhizoma 2 g, Ponciri Fructus Immaturus 3 g, Natrii Sulfas 3 g, Magnoliae Cortex 5 g	2 (11.1)

TABLE 2 Baseline demographic and clinical characteristics of patients.

Characteristics	Total ($n = 18$)[†]
Sex (Male/Female)	12 (66.7%)/6 (33.3%)
Age (year)	3.9 ± 0.9
Height (cm)	101.1 ± 8.6
Weight (kg)	17.1 ± 2.6
BMI (kg/cm^2)	22.0 ± 4.0
ADOS-2 (Autism/ASD)	10 (55.6%)/8 (44.4%)
ADOS-2	17.9 ± 8.1
CARS	34.6 ± 6.3

ADOS-2, autism diagnostic observation schedule, second edition; ASD, autism spectrum disorder; BMI, body mass index; CARS, childhood autism rating scale.
[†]Number (%) or mean ± standard deviation.

attention deficit hyperactivity disorder and schizophrenia, (3) those diagnosed with serious chronic disease, malignant tumors, and tuberculosis, (4) those who stopped taking risperidone or aripiprazole within 3 months of study initiation, and (5) patients who were judged to be inappropriate for participation in the research by the investigators (e.g., if no data were available for analysis because the evaluation was not performed on time).

Integrative treatment program

This 6-month integrative treatment program consisted of HM, Floortime, and SET. Individualized HM treatment was conducted based on the symptoms because this study was a pragmatic observational study with the goal of collecting information on the integrative treatment used in real-world clinical settings. Based on previous research and clinical experience, Shihogyeji-tang was used as the basis when sensory processing disorders were evident (19), Galgeun-tang when developmental dyspraxia was evident (20, 21), and Baekho-tang or Daeseunggi-tang when concentration disorders were evident (22, 23) (Table 1). Herbal medicine was prescribed for a total of 6 months, and the main prescriptions and dosages of individual herbs were changed, each month, according to patients' symptoms, and based on the judgment of a clinician with more than 20 years of clinical experience. Herbal medicine was administered in the form of a decoction, three times a day for a total volume of 40 ml per day. The type and dose of HMs used for individual patients were collected through case report forms (CRFs). Floortime was conducted for 1 h once a week with a certified professional therapist in consideration of the patient's individual developmental competency and profile. Parents were instructed to conduct Floortime with their children at home for more than 2 h a day, 5 days a week. Before the first session, all parents completed 12 h of an online training workshop from Floortime Center Korea (Seoul, Republic of Korea) to learn about Floortime. For SET, a certified professional therapist educated the parents once a week about patient-individualized counseling and play methods, including multi-sensory core training methods, such as smell, sight, touch, and taste. The parents were instructed to proceed with SET for more than 20 min a day (and on more than 5 days a week) at home with their children. The use of concomitant drugs was permitted if the investigator judged it necessary for patient treatment, and all drugs used during the observation period were recorded in the CRF.

Outcome measurement

The Korean versions of the Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) were used to assess the severity of the ASD symptoms, while the Social Maturity Scale (SMS) was used to assess social maturity, with three measurements at baseline, 3 and 6 months after treatment. The CARS is a validated questionnaire consisting of 15 items related to the core symptoms of ASD. It is a tool with high reliability, validity, and inter-rater consistency, with a total cutoff score of 30 that distinguishes ASD from other developmental disorders (24, 25). The ABC is a 57-item tool designed to distinguish children with ASD from normal children and children with intellectual disabilities, hearing impairments,

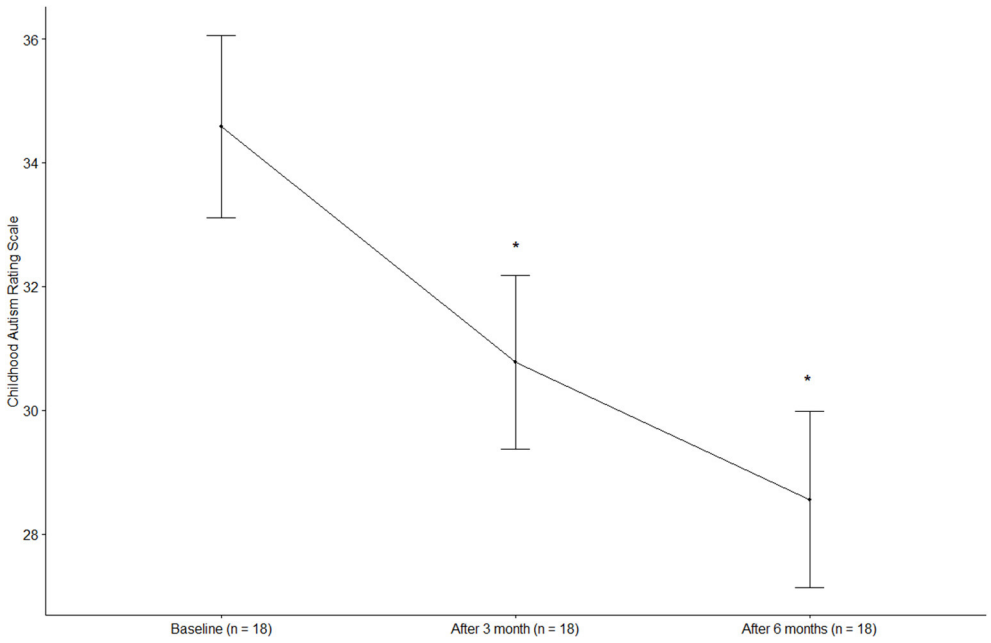


FIGURE 1
Changes in the childhood autism rating scale. *$p < 0.0001$, compared with baseline (Dunnett *post-hoc* test).

emotional disabilities. A total score of 67 or higher is classified as ASD (26). The SMS is a questionnaire consisting of 117 questions in 6 behavioral areas, including self-help, movement, work, communication, self-management, and socialization, and the social quotient is calculated based on the results (27). In addition, the Sequenced Language Scale for Infants (SELSI), a language evaluation tool, and ADOS-2, a tool for diagnosing ASD, were measured twice at baseline and 6 months after treatment by clinical psychologists. Symptom improvement is indicated by a low score in the case of CARS, ABC, and ADOS-2 but a high score for the SMS social quotient and SELSI. The ADOS-2 test results are classified into three categories according to the cut-off score: (1) autism, (2) ASD, or (3) non-ASD. According to these ADOS-2 classifications, responders are patients diagnosed with: (i) ASD or non-ASD after 6 months of treatment, following a diagnosis of baseline autism; or (ii) non-ASD after 6 months of treatment, following a diagnosis of baseline ASD. Assessment of the questionnaires used in this study was performed by the same qualified clinical psychologist or speech-language therapist, independent of the integrative treatment program.

For safety evaluation, adverse reactions were monitored based on physical examination and patient reports during the observation period, and the severity and causal relationship with treatment was recorded in detail in the CRF. Based on previous studies (28, 29), we considered the following as possible AEs related to HM: digestive system disorders (including indigestion, nausea, and diarrhea), nervous system disorders (including headache, fatigue, and dizziness), mental and behavior disorders (including loss of appetite, increased appetite, hypomania, and sedation), skin and subcutaneous tissue disorders (including rash and burning sensation of the skin), circulatory system disorders (including mild hypoglycemia, mild paroxysmal palpitation, and increased palpitation), respiratory system disorders (including upper respiratory tract infection), genitourinary system disorders (including uterine bleeding and dysuria), nutritional and metabolism disorders (including weight loss and dysgeusia), and others. Some AEs were considered by the investigator to have a causal relationship with the HM, based on the temporal relationship with treatment. Adverse events were classified as follows: mild (does not interfere with the patient's normal daily life and causes minimal discomfort), moderate (causes discomfort that significantly interferes with the patient's normal daily life), and severe (the patient's normal daily activities are impossible). Program adherence was calculated by collecting information from parents on performance rates for individual treatments, including HM, Floortime, and SET, during the observation period.

Statistical analysis

A linear mixed-effects model for repeated measures was used for assessing effect changes at baseline, 3 months,

and 6 months after treatment; a Dunnett *post-hoc* test was conducted for multi-group comparison. The effect changes at baseline and after 6 months were statistically analyzed using the two-sided paired *t*-test or Wilcoxon signed-rank test after a data normality test using a Shapiro-Wilk test. Categorical variables were expressed as frequency (percentage), and continuous variables were expressed as mean (standard deviation) or median (interquartile range) according to the normality of data. In addition, baseline characteristics predicting treatment responders were derived through logistic regression analysis, and univariable and multivariable odds ratios (ORs) and 95% confidence interval (CIs) were calculated. A p-value <0.05 was considered statistically significant. All statistical analysis was performed using R statistical software version 4.1.2 (for windows).

Results

Baseline characteristics

A total of 19 patients were screened for eligibility during the observation period, and a total of 18 patients were enrolled for the study, excluding one patient who was non-ASD on the ADOS-2 test. All enrolled patients participated in and completed the integrative treatment program for 6 months, and the treatment effect was evaluated every 3 months. Twelve of 18 patients (66.7%) were male, and the mean age and body mass index (BMI) of all patients were 3.9 ± 0.9 years and 22.0 ± 4.0, respectively. According to the ADOS-2 classification, 10 patients (55.6%) were identified to have autism, and 8 patients (44.4%) had ASD (Table 2). There was no patient with any clinically significant medical history or surgical history other than ASD. No patients received concomitant conventional medications, including risperidone, during the observation period.

Integrative treatment program and compliance rates

Herbal medicine was prescribed by a Korean medicine doctor and administered by parents three times a day for 6 months, according to the individual symptoms. Shihogyeji-tang- and Galgeun-tang-based prescriptions were used in 17 patients (each 94.4%), Baekho-tang-based prescription was used in 6 patients (33.3%), and Daeseunggi-tang based prescription was used in 2 patients (11.1%) (Table 1). The average compliance rate for HMs was 98.0% ± 4.4%. The parents of the patients were educated to perform Floortime and SET at least 5 times a week, for at least 2 h and 20 min a day, respectively. Based on this, the average performance rates of Floortime and SET were 94.9% ± 10.0% and 90.8% ± 23.3%, respectively.

Effect assessment

The CARS score was 34.58 ± 6.27 (mean ± standard deviation) at baseline and decreased significantly to 30.78 ± 5.95 after 3 months and 28.56 ± 6.05 after 6 months ($p < 0.0001$) (Figure 1). The total ABC score was 69.28 ± 15.73 at baseline and decreased significantly to 46.44 ± 19.14 after 3 months and 39.67 ± 20.36 after 6 months ($p < 0.0001$). The ABC subscales, including sensory, relating, body and object use, language, social, and self-help skills, all improved significantly at 3 and 6 months of treatment compared to the baseline ($p < 0.05$, all). The social quotient calculated by SMS after 6 months of treatment was higher than that at baseline ($p = 0.0627$). In the case of language ability measured by SELSI, both receptive and expressive language scores increased after treatment compared to the baseline, with a significant increase observed only in expressive language ($p = 0.0069$). The ADOS-2 score was significantly decreased after 6 months of treatment (12.39 ± 6.96) compared to the baseline (17.89 ± 8.11) ($p = 0.002$) (Table 3). After 6 months of treatment, the classification of ADOS-2 was as follows: autism in 3 patients (16.7%), ASD in 11 patients (61.1%), and non-ASD in 4 patients (22.2%). There were 9 (50%) treatment responders, as defined by the ADOS-2 classification change. In multivariate logistic regression analysis of the characteristics predicting treatment response, the lower the baseline CARS score, the higher was the treatment response (OR 0.65, 95% CI 0.30–0.98). Other characteristics such as age, sex, height, weight, BMI, and ADOS-2 score did not affect the treatment response (Table 4).

Safety assessment

During the observation period, a total of three AEs occurred in two patients: loose stool, constipation, and difficulty falling asleep. All AEs suspected of having a causal relationship with the HMs were mild because the AEs did not interfere with the patients' normal daily lives and caused minimal discomfort, and the AEs improved spontaneously without any treatment; therefore, these AEs were judged to have no effect on the study results.

Discussion

Autism spectrum disorder is a disease with increasing prevalence and a large social and economic burden. Behavioral therapy—the conventional treatment—is labor-intensive and costly, while there are concerns about the side effects of conventional medications. Therefore, the integrative treatment program, including HM, Floortime, and SET, has been used for many years in Korean medicine clinical practice. Therefore, we aimed to provide the first report of the effectiveness,

TABLE 3 Changes in outcome measures.

Outcomes	Baseline ($n = 18$)	After 3 months ($n = 18$)	After 6 months ($n = 18$)	p-values	
				Baseline vs. 3 months	Baseline vs. 6 months
CARS†	34.58 ± 6.27	30.78 ± 5.95	28.56 ± 6.05	<0.0001	<0.0001
ABC total†	69.28 ± 15.73	46.44 ± 19.14	39.67 ± 20.36	<0.0001	<0.0001
ABC sensory†	10.28 ± 3.95	5.00 ± 4.91	3.44 ± 3.57	<0.0001	<0.0001
ABC relating†	17.72 ± 6.69	10.22 ± 5.44	9.00 ± 6.41	<0.0001	<0.0001
ABC body and object use†	11.06 ± 5.10	8.06 ± 5.96	6.61 ± 5.91	0.0369	0.0011
ABC language†	18.61 ± 6.33	15.22 ± 6.69	12.17 ± 6.83	0.0015	<0.0001
ABC social and self-help†	11.61 ± 5.82	7.94 ± 3.84	8.44 ± 4.59	<0.0001	0.0003
SMS social quotient†	70.42 ± 15.79	73.82 ± 18.78	76.65 ± 26.26	0.4038	0.0627
SELSI receptive language score‡	26.00 (32.00)	–	29.50 (31.00)	–	0.0779
SELSI expressive language score†	27.67 ± 17.09	–	32.78 ± 16.10	–	0.0069
ADOS-2†	17.89 ± 8.11	–	12.39 ± 6.96	–	0.002

ABC, autism behavior checklist; ADOS-2, autism diagnostic observation schedule, second edition; CARS, childhood autism rating scale; SELSI, sequenced language scale for infants; SMS, social maturity scale.
† Mean ± standard deviation, ‡ median (interquartile).

TABLE 4 Logistic regression analysis: characteristics predicting treatment response.

Characteristics		Non-responder ($n = 9$)†	Responder ($n = 9$)†	Univariable OR (95% CI)	Multivariable OR (95% CI)
Age (year)		3.9 ± 0.9	4.0 ± 0.9	1.14 (0.37–3.71)	3.37 (0.45–76.74)
Sex	Female	2 (33.3%)	4 (66.7%)	–	–
	Male	7 (58.3%)	5 (41.7%)	0.36 (0.04–2.61)	1.34 (0.06–50.86)
Height (cm)		102.6 ± 7.4	99.6 ± 9.8	0.96 (0.84–1.07)	2.02 (0.52–12.34)
Weight (kg)		17.6 ± 2.7	16.7 ± 2.6	0.88 (0.58–1.27)	1.83 (0.71–7.92)
BMI (kg/cm^2)		21.2 ± 3.1	22.8 ± 4.8	1.12 (0.88–1.49)	8.44 (0.49–727.80)
ADOS-2		17.1 ± 9.8	18.7 ± 6.5	1.03 (0.91–1.17)	1.29 (0.97–2.10)
CARS		36.0 ± 5.9	33.2 ± 6.7	0.92 (0.76–1.08)	0.65 (0.30–0.98)

ADOS-2, autism diagnostic observation schedule, second edition; BMI, body mass index; CARS, childhood autism rating scale; CI, confidence interval; OR, odds ratio.
† Number (%) or mean ± standard deviation.

safety, and feasibility of the program in this prospective, observational study, and confirmed the predictive variables for the treatment response. Our study showed that a 6-month integrative treatment program resulted in a significant improvement of ASD symptoms and language ability, with a high program compliance rate and no serious AEs.

In particular, CARS and ABC scores dropped from respective averages of 34.58 and 69.28 at baseline to 28.56 and 39.67 after 6 months of treatment; both 6-month scores were below the ASD cut-off scores of 30 and 67, respectively (25, 26). Considering that the minimal clinically important difference for CARS score after an intervention has been defined as 4.5 points (30), an improvement of 6.02 points in CARS, which evaluates the core symptoms of ASD, after the integrative treatment program means a significant improvement. As a result of regression analysis of the patients' characteristics predicting the treatment response as defined by the ADOS-2 classification change, only the baseline CARS score was found to affect the treatment response. It was found that the lower the baseline CARS score, the higher was the treatment response. This means that if ASD is detected earlier and the integrative treatment program is implemented earlier, the treatment effect will be higher. This result is consistent with the current guidelines that emphasize

the importance of early detection of ASD and intensive treatment (31).

In this study, different basic HM prescriptions were used according to the main symptoms of each patient. Shihogyeji-tang, Galgeun-tang, and Baekho-tang or Daeseunggi-tang were used as the basic prescriptions for sensory processing disorder, developmental dyspraxia, and concentration disorder, respectively. In a previous study, the use of modified Shihogyeji-tang in 21 autistic patients resulted in a significant improvement in the sensory integration ability compared to before treatment (19). In addition, there have been studies showing the effect of Galgeun-tang on dystonia (20, 21) and of modified Daeseunggi-tang and Baekho-tang on attention deficit hyperactivity disorder (22, 23). It has been reported that functional emotional development and symptom severity were significantly improved when performing home-based Floortime for preschool children with ASD (32). Furthermore, several clinical trials have reported significant improvements in ASD severity, cognitive ability, sensory response, and language in the group receiving SET compared to children who did not receive SET (15, 33). Although the effects of individual treatments for the treatment of ASD have been determined by various researchers, to the best of our knowledge, our study is the first to report the effectiveness of this integrative treatment program. In addition, according to our study, program compliance was very high—>90% for all components—showing that it was sufficiently feasible in the clinical field.

Although research on the treatment mechanism of this integrative treatment program has not been conducted yet, the mechanisms of the individual treatments have been explored in several studies. The individual herbs of the HM prescriptions used in this program are used frequently for the treatment of ASD, ameliorate abnormal behavior in animal models of ASD, and have sedative and anticonvulsant effects (34–36). It has been reported that a shift occurs from the ventral to the dorsal systems in the medial prefrontal cortex in the brain of children with ASD after Floortime, suggesting that the children are gaining more cognitive control over their emotional system using such executive functions as response inhibition and self-monitoring (37). In addition, several animal studies have reported that SET changes the neuroplasticity of the brain. Sensory enrichment therapy resulted in rat cortical growth and an improvement in learning and motor memory (38). Furthermore, SET reduced neurological deficits, increased nerve dendrites, nerve branch density, nerve synapses, and surrounding blood vessels, as well as brain weight and size (39–42). Although the pathogenesis of ASD has not yet been clearly elucidated, recent studies have reported a link between abnormalities in brain regions and the core symptoms of ASD (43, 44). Therefore, it can be estimated that the therapeutic effect of this integrative treatment program was derived from the combination of these individual treatment mechanisms, warranting further research on the overall mechanism of the integrative treatment program.

This study has several limitations. First, since this study was a prospective observational study and not a controlled intervention study, it is not possible to draw clear conclusions about the reported effects. Although we confirmed that no other conventional medication was used during the observation period in the included patients, there may be other potential effects that could have affected the study outcome. Second, the safety assessment of the program relied solely on patient reports and physical examination. Considering that HMs were included in the treatment program, it would be helpful to report the safety of the program using laboratory tests in future studies. Finally, although adherence to individual treatment programs was >90%, on average, this was confirmed from parental reports. Because parents' adherence to the programs can have a great impact on study results, future research should aim to increase and systematically check parents' adherence.

Nevertheless, because conventional treatment for ASD is labor-intensive and expensive, and there are concerns about side effects of conventional medication, such an integrative treatment program would be highly beneficial. However, as there have been no studies on the effect of this integrative treatment program, we report the first case series on this integrative treatment program that is frequently used in Korean medicine clinical settings. We evaluated the therapeutic effect of HMs, Floortime, and SET on ASD using validated questionnaires and diagnostic tools, reduced related biases, and increased the reliability of the study results through a prospective observational study design. In particular, this is a treatment program in which the role of the parents is important, and as it can be conducted non-face-to-face in the "untact era," it is free from time and space constraints. In addition, there is a possibility that the therapeutic effect may be increased by increasing the role of the parents in performing Floortime and SET. Given the promising results of our study, further rigorous, well-designed controlled trials with a larger sample size should be conducted to confirm the clinical effectiveness of such integrative treatment programs.

Conclusion

A 6-month integrative treatment program, including HM, Floortime, and SET, could help improve the core symptoms of ASD with a high compliance rate and no serious AEs. Future well-designed, prospective randomized controlled trials with a larger sample size should be conducted to assess the effectiveness of the program.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of Gachon University (1044396-201812-HR-223-01). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MK: conception and design of the study. SP, HK, and MK: acquisition of data. BL: analysis of data and drafting the manuscript or figures. SP, HK, GH, and MK: critical review and edits. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Korea Institute of Oriental Medicine (KSN2022210).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: American Psychiatric Publishing. doi: 10.1176/appi.books.9780890425596

2. Maenner MJ, Shaw KA, Bakian AV, Bilder DA, Durkin MS, Esler A, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2018. *MMWR Surveill Summ.* (2021) 70:1–16. doi: 10.15585/mmwr.ss7011a1

3. Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. *Psychol Med.* (2015) 45:601–13. doi: 10.1017/S003329171400172X

4. Masi A, Demayo MM, Glozier N, Guastella AJ. An overview of autism spectrum disorder, heterogeneity and treatment options. *Neurosci Bull.* (2017) 33:183–93. doi: 10.1007/s12264-017-0100-y

5. Maneeton N, Maneeton B, Putthisri S, Woottiluk P, Narkpongphun A, Srisurapanont M. Risperidone for children and adolescents with autism spectrum disorder: a systematic review. *Neuropsychiatr Dis Treat.* (2018) 14:1811–20. doi: 10.2147/NDT.S151802

6. Owen-Smith AA, Bent S, Lynch FL, Coleman KJ, Yau VM, Pearson KA, et al. Prevalence and predictors of complementary and alternative medicine use in a large insured sample of children with autism spectrum disorders. *Res Autism Spectr Disord.* (2015) 17:40–51. doi: 10.1016/j.rasd.2015.05.002

7. Klein N, Kemper KJ. Integrative approaches to caring for children with autism. *Curr Probl Pediatr Adolesc Health Care.* (2016) 46:195–201. doi: 10.1016/j.cppeds.2015.12.004

8. Hanson E, Kalish LA, Bunce E, Curtis C, McDaniel S, Ware J, et al. Use of complementary and alternative medicine among children diagnosed with autism spectrum disorder. *J Autism Dev Disord.* (2007) 37:628–36. doi: 10.1007/s10803-006-0192-0

9. Bang M, Lee SH, Cho SH, Yu SA, Kim K, Lu HY, et al. Herbal medicine treatment for children with autism spectrum disorder: a systematic review. *Evid Based Complement Alternat Med.* (2017) 2017:8614680. doi: 10.1155/2017/8614680

10. Kasari C, Gulsrud AC, Wong C, Kwon S, Locke J. Randomized controlled caregiver mediated joint engagement intervention for toddlers with autism. *J Autism Dev Disord.* (2010) 40:1045–56. doi: 10.1007/s10803-010-0955-5

11. Schertz HH, Horn K, Lee M, Mitchell S. Supporting parents to help toddlers with autism risk make social connections. *Young Except Child.* (2017) 20:16–29. doi: 10.1177/1096250615576808

12. Green J, Wan MW, Guiraud J, Holsgrove S, McNally J, Slonims V, et al. Intervention for infants at risk of developing autism: a case series. *J Autism Dev Disord.* (2013) 43:2502–14. doi: 10.1007/s10803-013-1797-8

13. Sandbank M, Bottema-Beutel K, Crowley S, Cassidy M, Dunham K, Feldman JI, et al. Project AIM: autism intervention meta-analysis for studies of young children. *Psychol Bull.* (2020) 146:1–29. doi: 10.1037/bul0000215

14. Casenhiser DM, Shanker SG, Stieben J. Learning through interaction in children with autism: preliminary data from asocial-communication-based intervention. *Autism.* (2013) 17:220–41. doi: 10.1177/1362361311422052

15. Woo CC, Leon M. Environmental enrichment as an effective treatment for autism: a randomized controlled trial. *Behav Neurosci.* (2013) 127:487–97. doi: 10.1037/a0033010

16. Greenspan SI, Wieder S. A functional developmental approach to autism spectrum disorders. *J Assoc Pers Severe Handicaps.* (1999) 24:147–61. doi: 10.2511/rpsd.24.3.147

17. Aronoff E, Hillyer R, Leon M. Environmental enrichment therapy for autism: outcomes with increased access. *Neural Plast.* (2016) 2016:2734915. doi: 10.1155/2016/2734915

18. Krech D, Rosenzweig MR, Bennett EL. Effects of environmental complexity and training on brain chemistry. *J Comp Physiol Psychol.* (1960) 53:509. doi: 10.1037/h0045402

19. Li Y. Treatment of 21 cases of autism with the main formula of Chaihu Jiaguizhi Longgu Muli decoction. *Chin Med Mod Dist Educ China.* (2011) 9:57–8. doi: 10.3969/j.issn.1672-2779.2011.22.039

20. Wang G. Epidemic myodystonia syndrome treated by integrated traditional and western medicine: report of 88 cases. *J Tradit Chin Med.* (1994) 35:544–5.

21. Zhu E. One case of Meige syndrome treated by Gegen decoction. *Inner Mong J Tradit Chin Med.* (2018) 37:27–8. doi: 10.16040/j.cnki.cn15-1101.2018.02.021

22. Xiang H, Du Y. One case of infantile ADHD treated by modified Xiaochengqi decoction. *Guangxi J Tradit Chin Med.* (2008) 31:45.

23. Ding L, Li CJ, Jin D. Experience on the treatment for infantile attention-deficit hyperactivity disorder by Chinese medicine. *Chin Pediatr Integr Tradit West Med.* (2015) 7:502–4. doi: 10.3969/j.issn.1674-3865.2015.05.034

24. Schopler E, Reichler RJ, Devellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *J Autism Dev Disord.* (1980) 10:91–103. doi: 10.1007/BF02408436

25. Shin MS, Kim Y-H. Standardization study for the Korean version of the Childhood Autism Rating Scale: reliability, validity and cut-off score. *Kor J Clin Psychol.* (1998) 17:1–15.

26. Krug DA, Arick J, Almond P. Behavior checklist for identifying severely handicapped individuals with high levels of autistic behavior. *J Child Psychol Psychiatry.* (1980) 21:221–9. doi: 10.1111/j.1469-7610.1980.tb01797.x

27. Kim SG, Kim OG. *Social Maturity Scale Protocol*. Seoul: Jungang Aptitude Laboratory (1995).

28. Lee JY, Jun SA, Hong SS, Ahn YC, Lee DS, Son CG. Systematic review of adverse effects from herbal drugs reported in randomized controlled trials. *Phytother Res.* (2016) 30:1412–9. doi: 10.1002/ptr.5647

29. Jang S, Kim KH, Sun SH, Go HY, Lee EK, Jang BH, et al. Characteristics of herbal medicine users and adverse events experienced in south korea: a survey study. *Evid Based Complement Alternat Med.* (2017) 2017:4089019. doi: 10.1155/2017/4089019

30. Jurek L, Baltazar M, Gulati S, Novakovic N, Núñez M, Oakley J, et al. Response (minimum clinically relevant change) in ASD symptoms after an intervention according to CARS-2: consensus from an expert elicitation procedure. *Eur Child Adolesc Psychiatry.* (2022) 31:1–10. doi: 10.1007/s00787-021-01772-z

31. Sanchack KE, Thomas CA. Autism spectrum disorder: primary care principles. *Am Fam Physician.* (2016) 94:972–9.

32. Pajareya K, Nopmaneejumruslers K. A pilot randomized controlled trial of DIR/Floortime^{TM} parent training intervention for pre-school children with autistic spectrum disorders. *Autism.* (2011) 15:563–77. doi: 10.1177/1362361310386502

33. Woo CC, Donnelly JH, Steinberg-Epstein R, Leon M. Environmental enrichment as a therapy for autism: a clinical trial replication and extension. *Behav Neurosci.* (2015) 129:412–22. doi: 10.1037/bne0000068

34. Gonzales EL, Jang JH, Mabunga DF, Kim JW, Ko MJ, Cho KS, et al. Supplementation of Korean Red Ginseng improves behavior deviations in animal models of autism. *Food Nutr Res.* (2016) 60:29245. doi: 10.3402/fnr.v60.29245

35. Lee J, Son K, Hwang G, Kim M. Effect and safety of shihogyejitang for drug resistant childhood epilepsy. *Evid Based Complement Alternat Med.* (2016) 2016:3410213. doi: 10.1155/2016/3410213

36. Jiang H, Yang L, Hou A, Zhang J, Wang S, Man W, et al. Botany, traditional uses, phytochemistry, analytical methods, processing, pharmacology and pharmacokinetics of Bupleuri Radix: a systematic review. *Biomed Pharmacother.* (2020) 131:110679. doi: 10.1016/j.biopha.2020.110679

37. Stieben J, Lewis MD, Granic I, Zelazo PD, Segalowitz S, Pepler D. Neurophysiological mechanisms of emotion regulation for subtypes of externalizing children. *Dev Psychopathol.* (2007) 19:455–80. doi: 10.1017/S0954579407070228

38. Van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. *Nat Rev Neurosci.* (2000) 1:191–8. doi: 10.1038/35044558

39. Briones TL, Therrien B, Metzger B. Effects of environment on enhancing functional plasticity following cerebral ischemia. *Biol Res Nurs.* (2000) 1:299–309. doi: 10.1177/109980040000100406

40. Ben-Sasson A, Hen L, Fluss R, Cermak SA, Engel-Yeger B, Gal E. A meta-analysis of sensory modulation symptoms in individuals with autism spectrum disorders. *J Autism Dev Disord.* (2009) 39:1–11. doi: 10.1007/s10803-008-0593-3

41. Baroncelli L, Braschi C, Spolidoro M, Begenisic T, Sale A, Maffei L. Nurturing brain plasticity: impact of environmental enrichment. *Cell Death Differ.* (2010) 17:1092–103. doi: 10.1038/cdd.2009.193

42. Mering S, Jolkkonen J. Proper housing conditions in experimental stroke studies-special emphasis on environmental enrichment. *Front Neurosci.* (2015) 9:106. doi: 10.3389/fnins.2015.00106

43. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci.* (2008) 31:137–45. doi: 10.1016/j.tins.2007.12.005

44. Hazlett HC, Gu H, Munsell BC, Kim SH, Styner M, Wolff JJ, et al. Early brain development in infants at high risk for autism spectrum disorder. *Nature.* (2017) 542:348–51. doi: 10.1038/nature21369

frontiers | Frontiers in Neurology

TYPE Systematic Review
PUBLISHED 09 January 2023
DOI 10.3389/fneur.2022.1095444

OPEN ACCESS

EDITED BY
Lingyong Xiao,
First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China

REVIEWED BY
Bo Zhang,
Heilongjiang University of Chinese Medicine, China
Hongxuan Tong,
China Academy of Chinese Medical Sciences, China

*CORRESPONDENCE
Li Li
lily2015208@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 11 November 2022
ACCEPTED 12 December 2022
PUBLISHED 09 January 2023

CITATION
Li L, Huo B, Wang Y, Wang Y, Gong Y, Zhang Y, Liu T, Sha G and Zheng T (2023) Efficacy of Chinese herbal medicine on poststroke depression in animal models: A systematic review and meta-analysis.
Front. Neurol. 13:1095444.
doi: 10.3389/fneur.2022.1095444

Efficacy of Chinese herbal medicine on poststroke depression in animal models: A systematic review and meta-analysis

Li Li[1*†], Bixiu Huo[2†], Yuan Wang[1], Yao Wang[1], Ying Gong[3], Yun Zhang[1], Tingting Liu[1], Guiming Sha[1] and Tianru Zheng[1]

[1]Elderly Demonstration Ward, Beijing Geriatric Hospital, Beijing, China, [2]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]Department of Pharmacy, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, China

Background: Poststroke depression (PSD) is a common complication that can seriously affect patients' functional recovery and quality of life after a stroke. Various side effects have been found to be associated with the pharmacological therapies used for PSD. Studies have shown that Chinese herbal medicine (CHM) can effectively improve PSD-like behavior and neurological function in clinical and animal studies. The efficacy of CHM on PSD in animal models has not been systematically analyzed.

Methods: The following electronic databases were searched for articles published up to September 2022: PubMed, Web of Science, the Cochrane Library, and Embase. Studies that reported the efficacy of CHM in animals with PSD and were written in English were included. Depression-like behavior and the neurological deficit score were assessed as measures of efficacy. The included studies assessed depression-like behavior using sucrose preference, open-field, forced swimming, and tail suspension tests, as well as body weight. The Review Manager version 5.4 and STATA version 13.1 software packages were used for the meta-analysis. The standardized mean difference (SMD) with 95% confidence intervals was used to assess all the outcomes. Subgroup analyses were performed to explore the sources of heterogeneity. The Egger's test and funnel plots were used to assess the potential publication bias. Sensitivity analyses were used to identify the stability of the results.

Results: A total of 14 studies, including 12 CHMs involving 442 rats, fulfilled the inclusion criteria for meta-analysis. The pooled results showed that CHM significantly alleviated neurological deficits (−1.72 SMD, −2.47– −0.97) and was efficacious in improving the depression-like behavior of rats in the sucrose preference (2.08 SMD, 1.33–2.84), open-field (2.85 SMD, 1.88–3.83), forced swimming (−1.83 SMD, −2.23–1.44), and tail suspension tests (−1.35 SMD, −1.94–0.76).

Conclusion: Our results suggest that CHM could significantly improve depression-like behavior and neurological function in animals with PSD. The current results should be interpreted with caution because only animal studies were included.

KEYWORDS

animal model, Chinese herbal medicine, depression-like behavior, meta-analysis, post-stroke depression

Introduction

Stroke remains the second-leading cause of death and the third-leading cause of death and disability combined (as expressed by disability-adjusted life-years lost) in the world (1). Poststroke depression (PSD) is the most common psychiatric disorder following a stroke (2). The prevalence of PSD is approximately 33% (3, 4). A review reported that the incidence of PSD within 2 years after stroke ranges from 11% to 41%. PSD is similar to major depressive disorder (MDD) but some symptoms differ (5, 6). Patients with PSD generally present with mood fluctuations, irritability, or apathy, while anhedonia, pessimism, suicidal ideation, or attention deficits are more common in patients with MDD. PSD negatively impacts rehabilitation following stroke, significantly increasing the chances of relapsing neurovascular events (3). PSD severely restricts the ability of patients to care for themselves and increases their dependence on other people in activities of daily living, which leads to poor quality of life (7). Moreover, PSD is associated with a significantly increased mortality risk in stroke survivors. A systematic review that included 15 prospective cohort studies, 250,294 participants, and 139,276 cases assessed the association between PSD and the risk of death and concluded that PSD increases all-cause mortality by 59% (8). Furthermore, the healthcare cost of patients with PSD is about four times that of poststroke patients without depression (9). Therefore, it is clear that PSD has serious economic impacts.

The pathophysiology of PSD is multifactorial and unclear. It probably involves dysregulation of the hypothalamic-pituitary-adrenal axis, increased inflammatory factors, decreased levels of monoamines, glutamate-mediated excitotoxicity, and abnormal neurotrophic response (10). The treatments for PSD include medication, psychotherapy, social intervention, and repetitive transcranial magnetic stimulation (11). Tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors, and monoamine oxidase inhibitors are the most common antidepressant drugs used (10). SSRIs are the first-choice drugs used to treat PSD, and tricyclic drugs should be considered for second-line therapy (4). However, almost all antidepressant drugs are associated with adverse effects. A large cohort study suggested that all commonly used antidepressants can contribute to significantly increased risks of adverse outcomes, including mortality, suicide, hemorrhagic complications, falls, and upper gastrointestinal bleeding (12–14). High-quality evidence suggests that SSRIs increased the risk of bone fractures and possibly the risk of seizures (15). Therefore, other treatment options need to be explored.

Traditional Chinese medicine, including Chinese herbal medicine (CHM), acupuncture, moxibustion, and Tuina, has long been used to manage various disorders in China (16). A series of animal studies (17–21) have shown that traditional Chinese medicine can improve depression-like behavior and neurological function of PSD rats by regulating the signaling pathways in the neurotrophic pathway and reducing neuroinflammatory responses. However, the efficacy of CHM in PSD animals has not been systematically reviewed. Here, we performed a systematic review and meta-analysis to comprehensively review the efficacy of CHM in treating PSD based on animal studies.

Methods

We performed this systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (22). The efficacy outcomes assessed include the neurological deficit score, body weight, as well as sucrose preference, open-field, forced swimming, and tail suspension test results. Low body weight, less sugar consumption in the sucrose preference test, longer immobility in the forced swimming and tail suspension tests, and less movement in the open-field test were considered to be signs of depression.

Search strategy

Studies published up to 25 September 2022 were retrieved from the Web of Science, PubMed, Cochrane Library, and Embase databases. Studies that reported the efficacy of

CHM in animals with PSD were included. The literature search was conducted using the terms ("depression") AND ("stroke") AND ("animal" OR "animals" OR "rat" OR "rats" OR "mouse" OR "mice") AND ("Chinese medicine" OR "Chinese herb" OR "herbal" OR "natural drug" OR "Natural Product" OR "traditional Chinese" OR "formula"). Only publications written in English were included in this review. All searches were performed by two independent researchers (TL and GS).

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) studies that reported the efficacy of CHM in PSD animal models; (2) studies that used CHM, including Chinese herbal compounds, a single Chinese herbal extract (monomer), and compounds of several Chinese herbal extracts; (3) studies that mentioned the number of animals used; and (4) studies that were published in English.

The exclusion criteria were as follows: (1) studies that included an agent that was a natural plant extract but not the main ingredient in traditional CHM; (2) studies where the data could not be obtained. Studies were independently screened by two researchers (LL and YG), and any disagreements were resolved through discussion.

Data extraction

Two investigators (LL and BH) independently extracted data from the included studies. We extracted data on the outcome measures (neurological deficit score and body weight as well as the sucrose preference, open-field, forced swimming, and tail suspension test results), publication details (author and year), treatments used (route and dose), and animals (species) from the studies. For each comparison, the sample size, mean value, and standard deviation (SD)/standard error for both the treatment and control groups were extracted. If multiple treatment groups shared the same control group, the sample size of the control group was divided by the number of treatment groups (23). If outcomes were measured at more than one time point, we only included data from the last time point (24). If outcomes were presented graphically, we used the ImageJ software (NIH, United States) to quantify the results.

Risk of bias assessment

Two researchers independently assessed the quality of the included studies using the Systematic Review Center for Laboratory Animal Experimentation Risk of Bias tool. The assessment covered 10 areas, and each item was scored as one point as follows: (1) sequence generation, (2) baseline characteristics, (3) allocation concealment, (4) random housing,

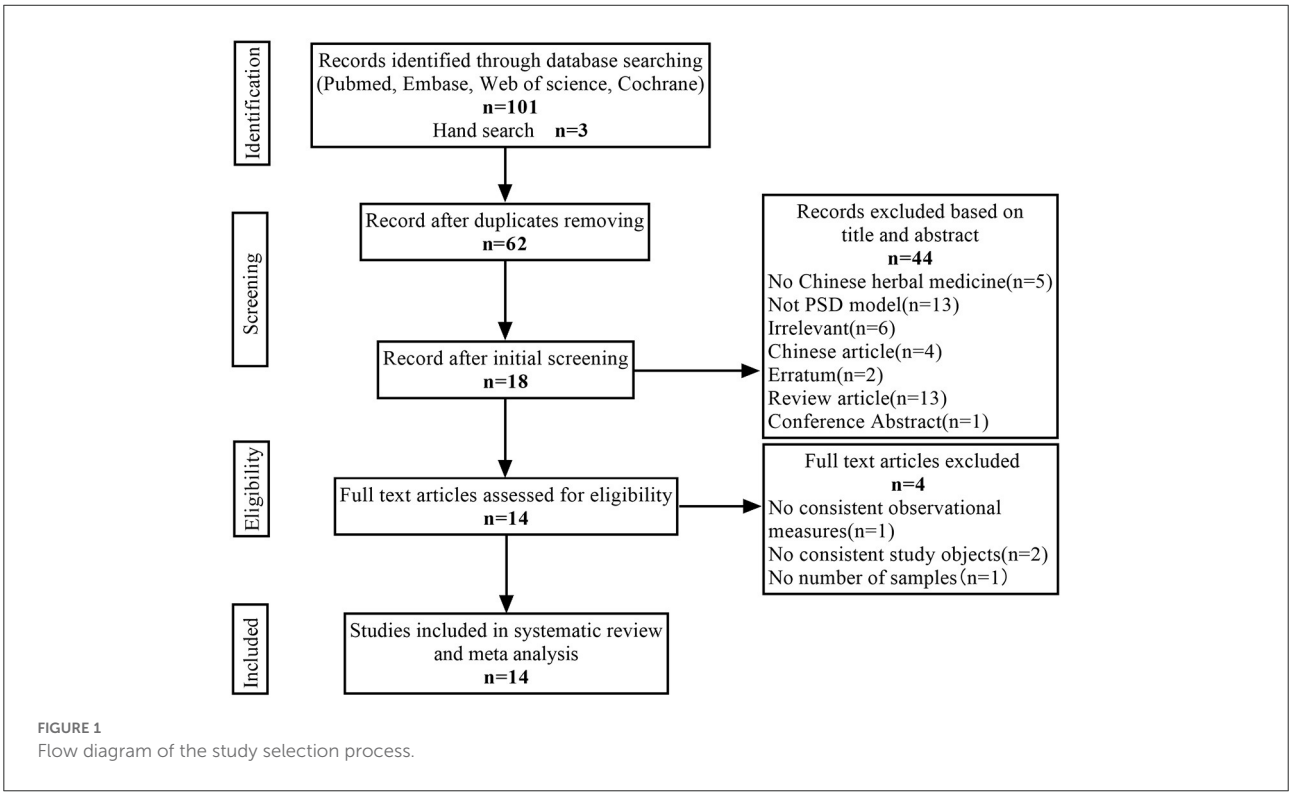


FIGURE 1
Flow diagram of the study selection process.

TABLE 1 Characteristicsof included studies.

References	CHM drug	Route	Duration (days)	Species	Weight (g)	Age (weeks)	Model	*N* (T/C)	Treat	Control	Possible mechanisms	Outcome measures
Du et al. (27)	JYAS	i.g.	28	SD rats	240–260	7–9	MCAO+CUMS	10/10	1g/kg/d 3g/kg/d	NR	Modulation on monoamine system, neuroendocrine, neuroinflammation, and neurogenesis	SPT↘OFT
Fan et al. (28)	XNJY	i.g,	21	SD rats	NR	24	unilateral carotid artery ligation +separately housed +reserpine	10/10	45 mg/100g/d 15 mg/100g/d 7.5mg/100g/d	Saline	Increased the size and quantity of hippocampal neurons and glial cells with regular shape and decreased intercellular space	SPT↘OFT
Li et al. (18)	XNJY	i.g,	21	SD rats	180 ± 20	NR	MCAO+CUMS	12/12	10.5g/kg/d 21g/kg/d	NR	Regulation of the BDNF/ERK/CREB signaling pathway.	BW↘SPT OFT↘FST
Li et al. (19)	MOOs	Orally	14	SD rats	250–280	NR	tMCAO+CUMS	8/8	0.1 mg/g/d	Vehicle	Negatively regulate the microglial NLRP3 inflammasome activation	BW↘SPT OFT↘FST↘ TST
Tian et al. (29)	YNJYP	Gavage	56	SD rats	300–320	7–9	MCAO+CUMS	6/6	9.92 g/kg/d	Saline	Dynamically regulating the expression of Notch signaling genes	SPT↘FST
Yan et al. (30)	XNJY	i.g,	28	SD rats	175 ± 20	NR	unilateral internal carotid artery ligation+solitary housing +CUMS	8/8	45mg/100g/d 15mg/100g/d 7.5mg/100g/d	NR	Upregulate synaptotagmin expression in hippocampi	SPT↘OFT
Wang et al. (21)	SSA	i.p.	24	SD rats	230–260	NR	MCAO + isolation + CUMS	10/10	5 mg/kg	Saline	Inhibited hippocampal neuronal apoptosis after cerebral ischemia through p-CREB/ BDNF pathway	BW↘NDS↘ SPT↘OFT↘ FST
Zhao et al. (31)	JDTLG	i.g.	28	SD rats	200–220	NR	carotid artery embolization+chronic sleep deprivation	10/10	2 g /kg/d 4 g /kg/d	Water	Reduce glutamate (Glu) level and increase gamma-aminobutyric acid (GABA) level via regulating the NMDAR/BDNF pathway	BW↘NDS↘ SPT↘OFT↘ TST

(Continued)

10.3389/fneur.2022.1095444

TABLE 1 (Continued)

References	CHM drug	Route	Duration (days)	Species	Weight (g)	Age (weeks)	Model	*N* (T/C)	Treat	Control	Possible mechanisms	Outcome measures
Zhao et al. (32)	YNJYD	Gavage	28	Wistar rats	200 ± 10	6	MCAOS+CUMS	6/6	0.4 g/kg/d	Saline	Improving microcirculation in the PFC and HP, regulating glutamatergic systems and membrane phospholipid metabolism, and repairing microstructural damage	SPT↘OFT
Zhu et al. (17)	MOOs	Orally	14	SD rats	250-280	NR	tMCAO+CUMS	8/8	0.1 mg/g/d	Vehicle	Upregulating GLUT3 to improve synaptic activity	BW↘SPT↘ OFT↘FST↘ TST
Wang et al. (33)	BSYQ HXQY DTKQ	i.g.	56	Wistar rats	250±50	NR	Deligating bilateral common carotid arteries permanently+forced swimming	8/8	BSYQ 18g/kg/d HXHY 9g/kg/d, DTKQ 9g/kg/d)	Saline	Regulating the expressions of c-Fos and c-Jun in hippocampus	OFT
Yan et al. (34)	XNJY	i.g.	21	SD rats	200 ± 20	NR	MCAOS+CUMS	12/12	10.5g/kg/d 21g/kg/d 42g/kg/d	Saline	Alleviating neuroinflammation	BW↘SPT↘ OFT↘FST
Hu et al. (35)	PF	i.p.	21	SD rats	240–260	NR	MCAOS+CUMS	46/46	5mg/kg/d	Saline	Increased BDNF and p-CREB expression in the CA1 region	BW↘NDS↘ SPT↘OFT
Du et al. (36)	HupA	i.g.	28	SD rats	240–260	NR	MCAO+CUMS	8/10	0.05 mg/kg/d 0.15 mg/kg/d	NR	Upregulate hippocampal expression of 5-HT1AR, p-CREB and BDNF, and increase levels of NE, DA, and 5-HT in the hippocampus and prefrontal cortex	NDS↘SPT↘ FST

JYAS, Jieyu Anshen; XNJY, Xingnao Jieyu; MOOs, Morinda officinalis oligosaccharides; YNJYP, Yi-nao-jie-yu Prescription; SSA, Saikosaponin A; JDTLG, Jiedu Tongluo Granules; YNJYD, Yi-nao-jie-yu Decoction; BSYQ, Bushen Yiqi; HXQY, Huoxue Huayu; DTKQ, Ditan Kaiqiao; PF, paeoniflorin; HupA, Huperzine A; i.g., intragastric injection; i.p., intraperitoneal injection; SD, Sprague Dawley; NR, not reported; MCAO, middle cerebral artery occlusions; tMCAO, transient middle cerebral artery occlusion; CUMS, chronic unpredictable mild stress; d, days; BW, body weight; NDS, neurological deficit score SPT, sucrose preference test; FST, forced swimming test; OFT, open field test; TST, tail suspension test; BDNF, brain-derived neurotrophic factor;EPK, extracellular signal-regulated kinase; CREB, cyclic adenosine monophosphate response element binding protein; NLRP-3, Nucleotide- binding domain leucine- rich repeat family pyrindomain containing 3; p-CREB, phosphorylated CREB; NMDAR, N-methyl-D-aspartate receptors; GLUT3, glucose transporter-3; 5-HT_{1A}R, 5-hydroxytryptamine 1A receptor;NE, norepinephrine; DA, dopamine; 5-HT, 5-hydroxytryptamine.

10.3389/fneur.2022.1095444

(5) blinding (for animal breeders and researchers), (6) random outcome assessment, (7) blinding (for the outcome evaluator), (8) incomplete outcome data, (9) selective outcome reporting, and (10) other sources of bias. Any disagreements arising from the evaluation were resolved through discussion until a consensus was reached.

Subgroup analysis

There were no significant differences in the subjects, modeling, and administration methods used in the included studies. While the CHM used differed, it could be grouped into monomers and compounds. We performed a subgroup analysis on the different types (according to name) and forms (compound or monomer) of CHM to evaluate the influence of the variables or research characteristics on the estimated effect size.

Data analysis

Depending on the heterogeneity test, data were synthesized using a fixed effect model or random effect model. Heterogeneity across studies was explored by Cochran's Q statistic and I^2 statistic (25). The effect size was pooled using a random-effects model for the neurological deficit score, body weight, sucrose preference test results, and open-field test results. For the measured values of the forced swimming and tail suspension tests, the effect sizes were pooled using the fixed-effects model. We established a subgroup to explore the source of heterogeneity. The Egger's test and funnel plots (26) were used to assess the potential publication bias. Sensitivity analysis, performed by removing individual studies one at a time, was used to assess the stability of the results. All the data were analyzed using the Review Manager version 5.4 and STATA version 13.1 software packages.

Results

Study characteristics

A total of 104 articles (20 from PubMed, 20 from Web of Science, 20 from Cochrane Library, 41 from Embase, and 3 from hand search) were found, 42 duplicate articles were excluded using Endnote X 9, and 44 articles were manually excluded according to the title and abstract. For the remaining 18 articles, four articles that did not meet the inclusion criteria were excluded after reading the full text. Therefore, 14 articles were finally included in our analysis. The specific screening procedure is shown in Figure 1.

Altogether, 442 animals (278 in the CHM group; 164 in the PSD group) were included in this meta-analysis. Twelve studies used Sprague Dawley rats, and two studies used Wistar rats. The weight of the rats varied between 155 and 320 g. Only four studies mentioned the age of the rats, which ranged from 7 to 24 weeks. There were five kinds of PSD models used in the included studies. Ten studies used middle cerebral artery occlusion combined with chronic unpredictable mild stress (CUMS). One

TABLE 2 Study quality score report.

Author	Year	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	Aggregate quality score
Du, Y	2020	–	+	–	+	–	–	–	+	+	+	**5**
Fan, WT	2012	–	+	–	+	–	+	–	+	+	+	**6**
Li, T	2018	–	+	–	+	–	–	+	+	+	+	**6**
Li, ZF	2021	–	+	–	+	–	+	–	+	+	+	**6**
Tian, HL	2018	–	+	–	+	–	–	–	+	+	+	**5**
Yan, YM	2013	–	+	–	+	–	+	–	+	+	+	**6**
Wang, AR	2021	–	+	–	+	–	+	+	+	+	+	**7**
Zhao, AM	2021	–	+	–	+	–	+	–	+	+	+	**6**
Zhao, ZJ	2020	–	+	–	+	–	+	+	+	+	+	**7**
Zhu, JY	2020	–	+	–	+	–	+	+	+	+	+	**7**
Wang, HY	2006	–	+	–	+	–	+	–	+	+	+	**6**
Yan, YM	2019	–	+	–	+	–	+	+	+	+	+	**7**
Hu, MZ	2019	–	+	–	+	–	+	+	+	+	+	**7**
Du, Y	2017	–	+	–	+	–	+	+	+	+	+	**7**

"+" means this part was reported in the study, "–" means not reported.

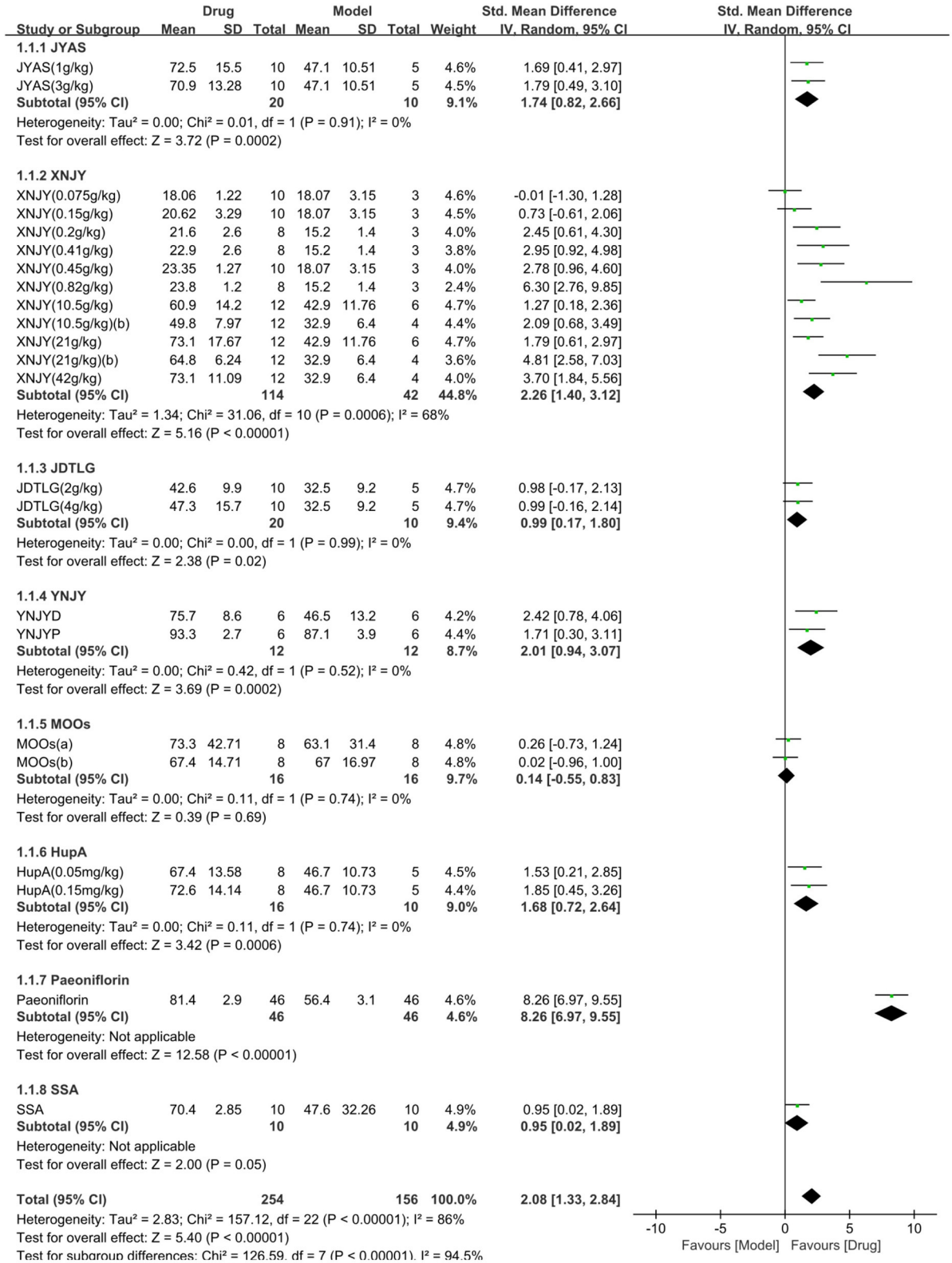


FIGURE 2
Efficacy of Chinese herbal medicine (CHM) on sucrose preference test. Forest plots of the effect size calculated using standardized mean differences (SMDs). The horizontal error bars represent the 95% confidence interval of individual studies.

study used unilateral carotid artery ligation combined with separate housing and a small dose of reserpine. One study used unilateral internal carotid artery ligation combined with solitary housing and CUMS. One study used carotid artery embolization combined with chronic sleep deprivation. One study used bilateral common carotid artery ligation combined with forced swimming.

Regarding the intervention methods, there were 12 types of CHM, including eight compounds and four monomers. The monomers included Morinda officinalis oligosaccharides (MOOs), peoniflorin, Saikosaponin A (SSA), and Huperzine A (HupA). The intervention durations ranged from 14 to 56 days. Intraperitoneal administration was performed in 2 of the 14 studies, and gastrointestinal administration was performed in the other 12 studies. Among the 14 included studies, 13, 12, 7, and 3 performed the sucrose preference, open-field, forced swimming test, and tail suspension tests, respectively, and seven and four measured body weight and neurological deficit scores, respectively. Among the 12 studies that conducted an open-field test, the effect sizes of three studies were inconsistent with the other nine studies. Therefore, we chose to analyze only nine of them. The detailed study characteristics are listed in Table 1.

Risk of bias and quality of the included studies

Among the 14 included studies, no study described the method used to generate the allocation sequence. All studies reported similar baseline characteristics between the groups. No

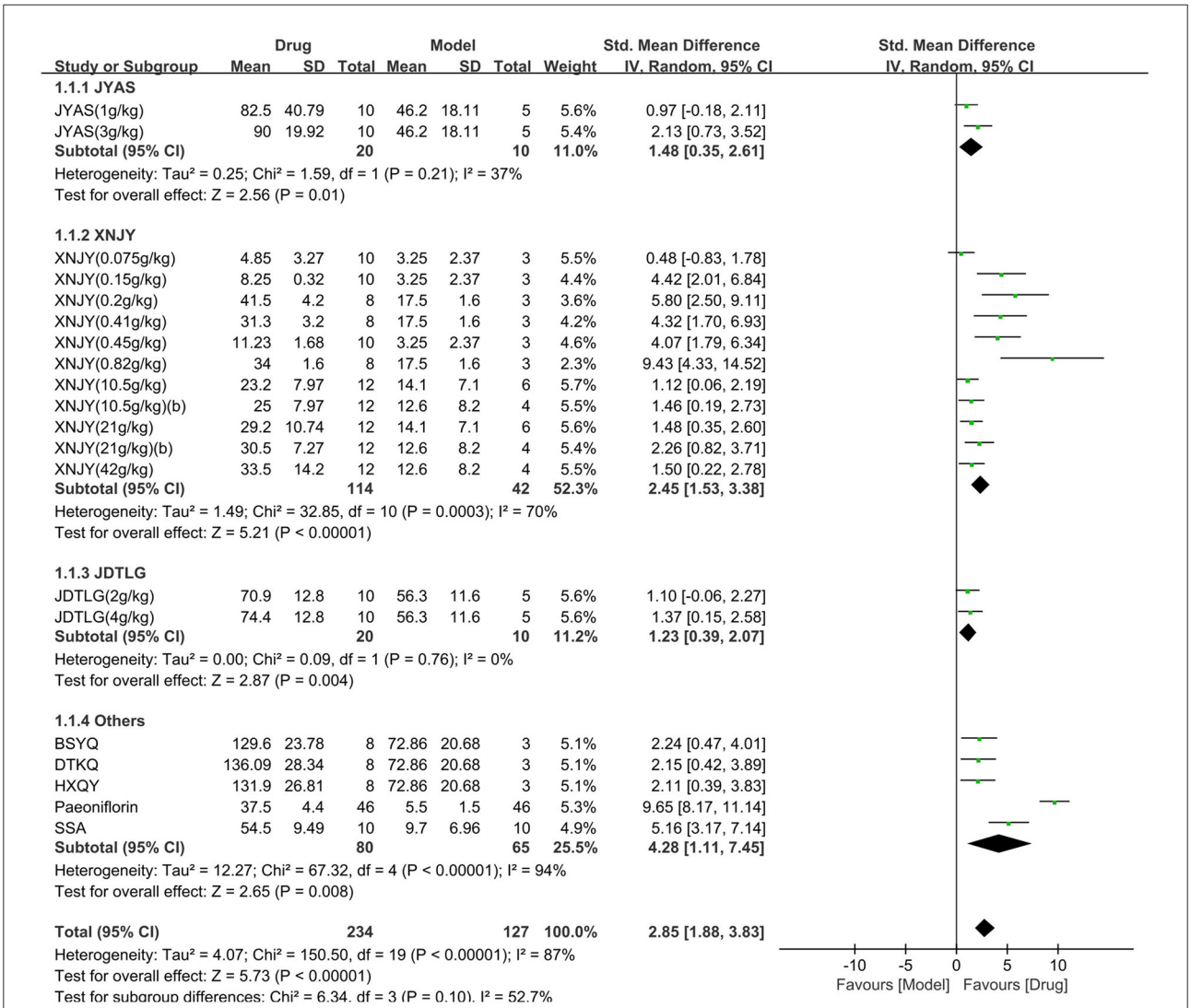


FIGURE 3
Efficacy of CHM on the open-field test. Forest plots of the effect size calculated using SMDs. The horizontal error bars represent the 95% confidence interval of individual studies.

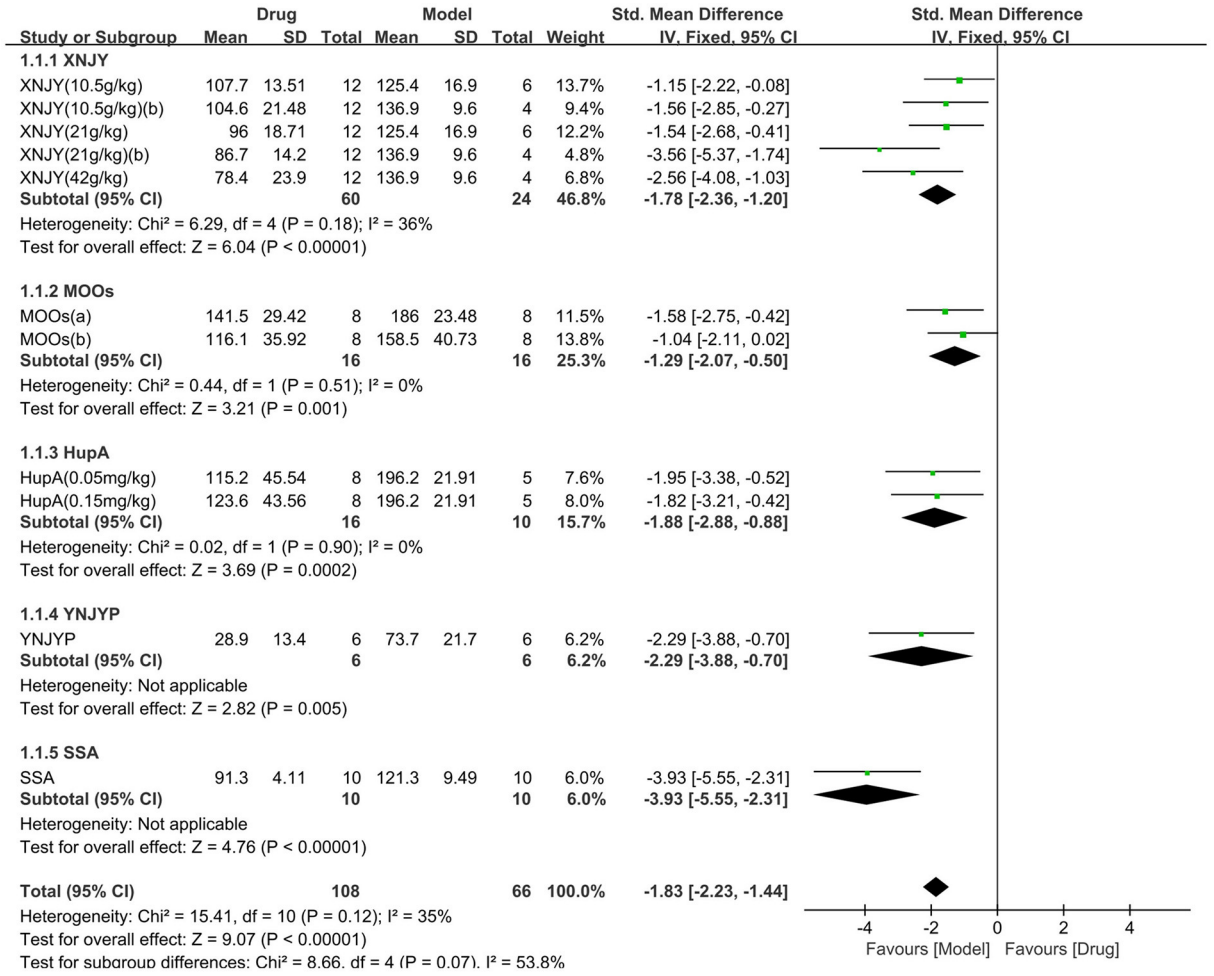


FIGURE 4
Efficacy of CHM on forced swimming test. Forest plots of the effect size calculated using SMDs. The horizontal error bars represent the 95% confidence interval of individual studies.

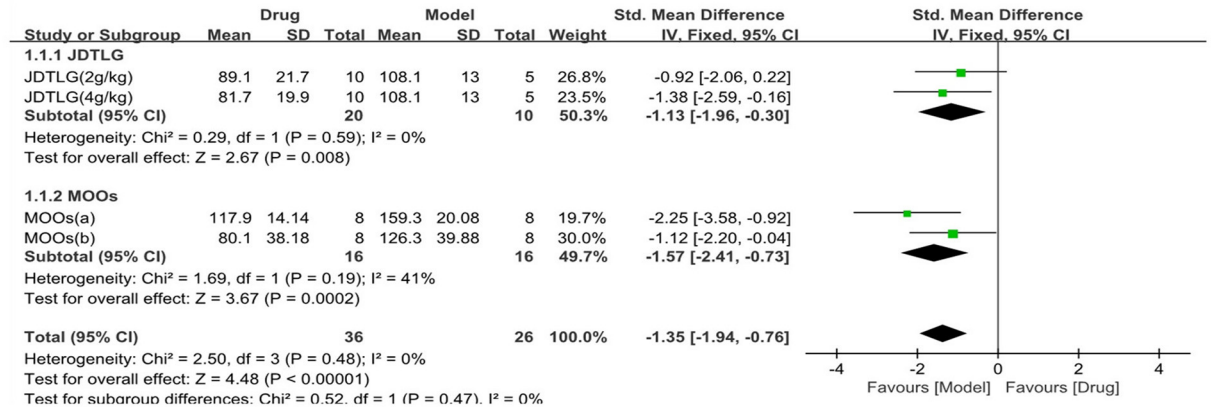


FIGURE 5
Efficacy of CHM on tail suspension test. Forest plots of the effect size calculated using SMDs. The horizontal error bars represent the 95% confidence interval of individual studies.

study clarified whether the allocation of different groups was sufficiently blinded. The breeding conditions and environments of all experimental animals included in the studies were the same. Therefore, we considered that the animal placements complied with the principle of randomization. No study reported sufficient information regarding the blinding methods used by the caregivers or investigators. A total of 11 studies assessed the randomization of the outcomes. Seven studies described the blinding methods used for the outcome evaluation. All studies reported complete outcome data and preliminary reported results and had no other sources of bias. A complete quality assessment of the included studies is shown in Table 2.

Meta-analysis on the effects of CHM

Effects on depression-like behavior

The sucrose preference test was used in 13 studies (410 animals, 254 were in the CHM group and 156 were in the control group), which are included in the meta-analysis (Figure 2). The pooled results showed that CHM significantly increased sucrose preference by 2.08 SMD (95% CI, 1.33–2.84; $P < 0.001$; 23 comparisons), with substantial heterogeneity between studies ($\chi^2 = 157.12$; $I^2 = 86\%$; df = 22; $P < 0.001$). Subgroup analysis showed a significant correlation ($P < 0.001$). The effects of MOOs and SSA were similar to that of the control group ($P = 0.69$, $P = 0.05$, respectively). The effects of the other CHM on the sucrose preference test were more obvious than that of the control group ($P < 0.05$). The forest map showed that peoniflorin played a more prominent role.

The open-field test was used in nine studies (361 animals, 234 were in the CHM group and 127 were in the control group), which are included in the meta-analysis (Figure 3). The pooled results showed that the CHM group had significantly improved the movement in the open-field test compared to the control group by 2.85 SMD (95% CI, 1.88–3.83; $P < 0.001$; 20 comparisons), with substantial heterogeneity between studies

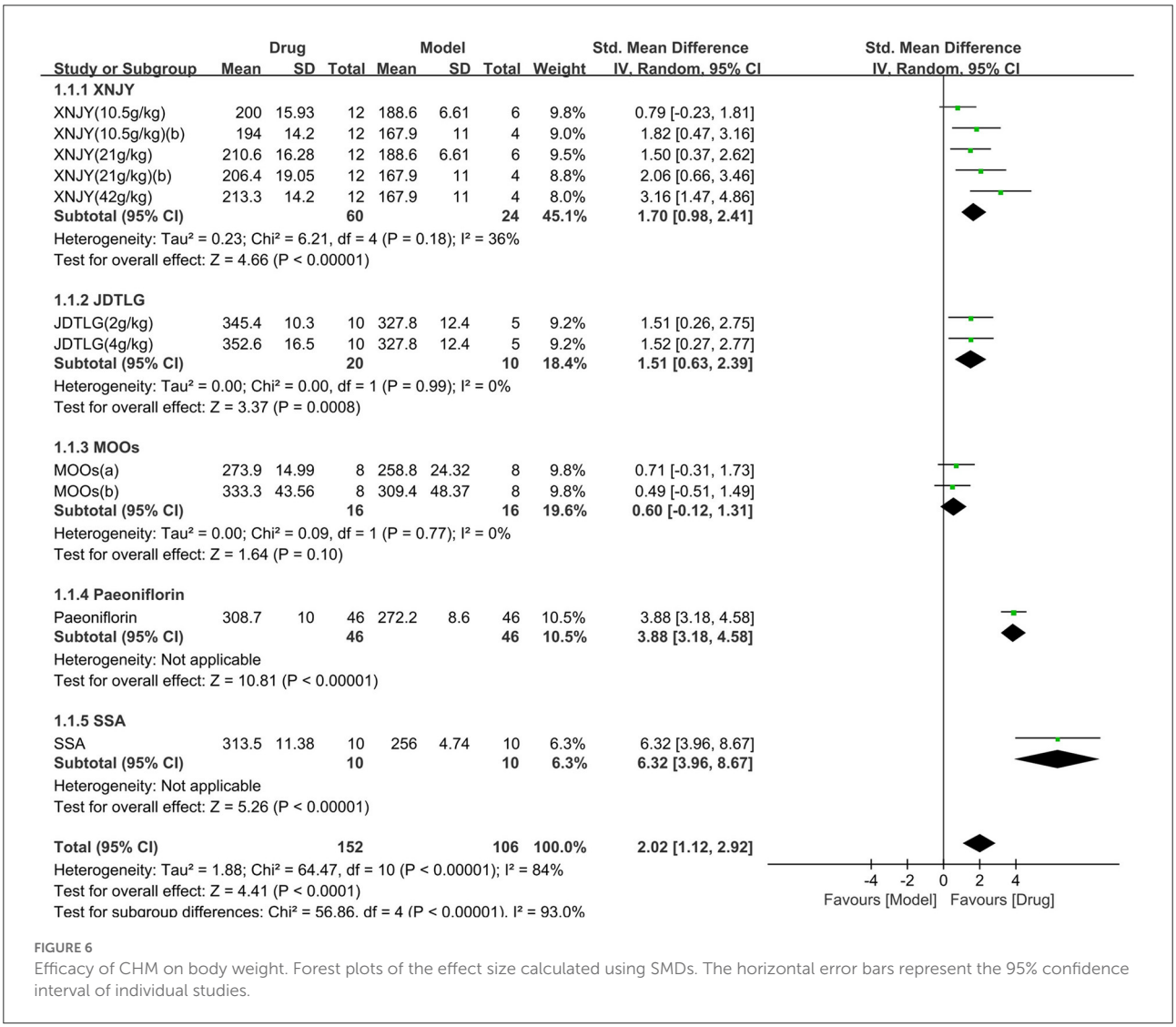


FIGURE 6
Efficacy of CHM on body weight. Forest plots of the effect size calculated using SMDs. The horizontal error bars represent the 95% confidence interval of individual studies.

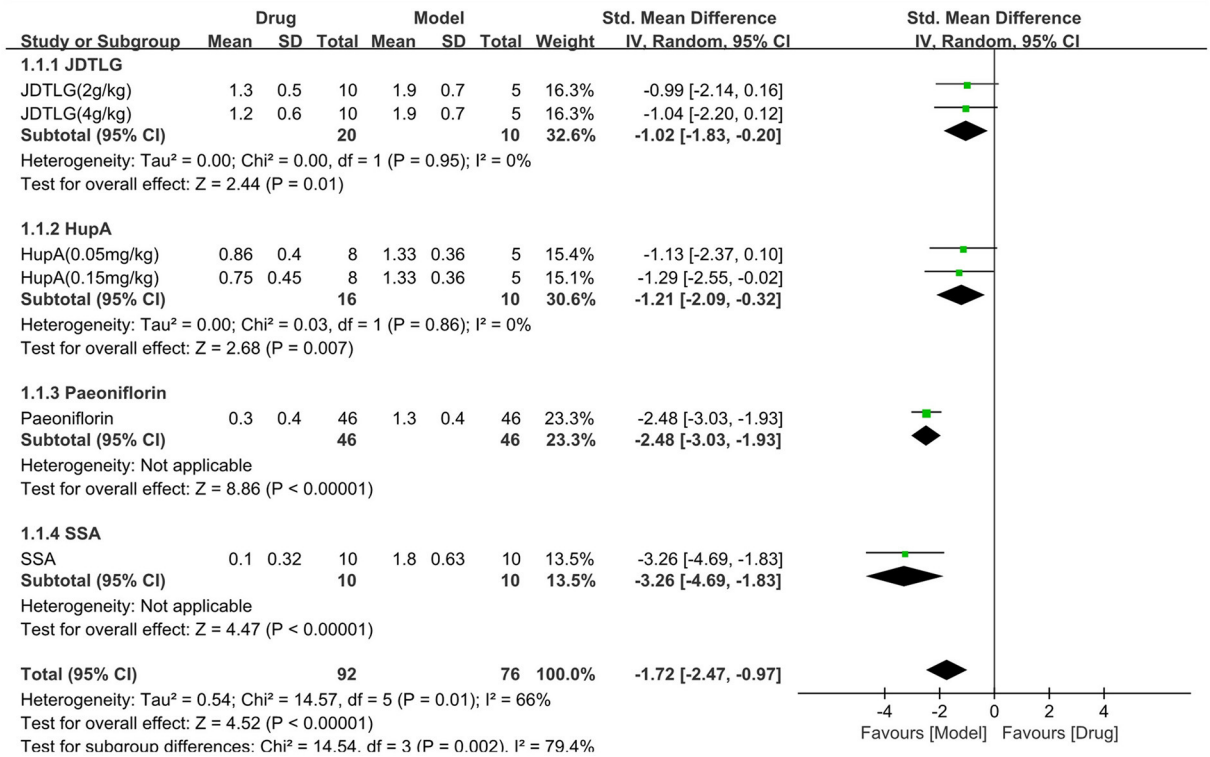


FIGURE 7
Efficacy of CHM on neurological deficit score. Forest plots of the effect size calculated using SMDs. The horizontal error bars represent the 95% confidence interval of individual studies.

(χ^2= 150.5; I^2= 87%; df = 19; $P < 0.001$). Subgroup analysis did not show any effect of the type of CHM on the results ($P = 0.1$), and each drug improved the movement in the open-field test ($P < 0.05$).

As for the forced swimming test, seven studies (174 animals, 108 were in the CHM group and 66 were in the control group) were included in the meta-analysis (Figure 4). The pooled results showed that CHM significantly decreased the immobility time of the forced swimming test by −1.83 SMD (95% CI, −2.23−1.44; $P < 0.00001$; 11 comparisons), with moderate heterogeneity between studies (χ^2= 15.41; I^2= 35%; df = 10; $P = 0.12$). No statistical significance was found when the CHM type was analyzed as a subgroup ($P = 0.07$), and each drug reduced the immobility time of the forced swimming test ($P < 0.05$). The forest map showed that SSA played a more prominent role.

The tail suspension test was used in three studies (62 animals, 36 were in the CHM group and 26 were in the control group), which are included in the meta-analysis (Figure 5). The pooled results showed that the immobility time in the tail suspension test was significantly decreased in the CHM group compared to the control group by −1.35 SMD (95% CI, −1.94 to −0.76; $P < 0.001$; 4 comparisons), with no heterogeneity between studies (χ^2= 2.5; I^2= 0; df = 3; $P = 0.48$). Subgroup analysis did not show any effect of the type of CHM on the results ($P = 0.47$), and each drug reduced the immobility time in the tail suspension test ($P < 0.05$).

Body weight was assessed in seven studies (258 animals, 152 were in the CHM group and 106 were in the control group), which are included in the meta-analysis (Figure 6). The pooled results showed that CHM significantly increased body weight by 2.02 SMD (95% CI, 1.12–2.92; $P < 0.001$; 11 comparisons), with substantial heterogeneity between studies (χ^2= 64.47; I^2= 84%; df = 10; $P < 0.001$). Subgroup analysis showed a significant correlation between the type of CHM and effect size ($P < 0.001$). The effect of MOOs on body weight was similar to the control group ($P = 0.1$), but the remaining CHM were all associated with significantly increased body weight ($P < 0.05$). The forest map showed that the effect of SSA and peoniflorin remained more obvious.

Effect on neurological deficit score

The neurological deficit score was assessed in four studies (168 animals, 92 were in the CHM group and 76 were in the control group), which are included in the meta-analysis (Figure 7). The pooled results showed that the neurological

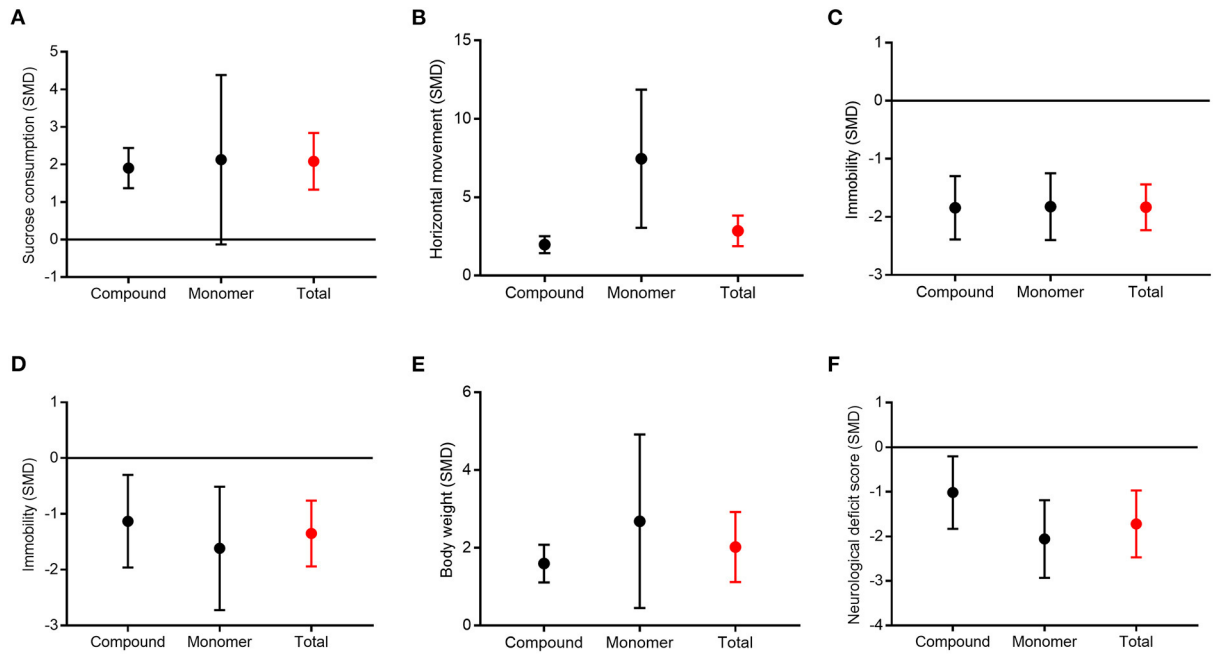


FIGURE 8
Subgroup analysis between compound and monomer of CHM. Sucrose preference test **(A)**, open-field test **(B)**, forced swimming test **(C)**, tail suspension test **(D)**, body weight **(E)**, and neurological deficit score **(F)**.

deficit score of the CHM group was significantly lower than that of the control group by −1.72 SMD (95% CI, −2.47−0.97; $P <$ 0.001; 6 comparisons), with substantial heterogeneity between studies ($\chi^2 = 14.57$; $I^2 = 66\%$; df = 5; $P = 0.01$). Subgroup analysis was performed according to the type of CHM and showed a significant correlation between the type of CHM and effect size ($P = 0.002$). The forest map showed that the effect of SSA and peoniflorin seemed to be more obvious, although each drug improved the neurological deficit score ($P < 0.05$).

Subgroup analysis between compound and monomer CHM

Due to the high heterogeneity among the meta-analyses, we further conducted a subgroup analysis according to the form of CHM (compound vs. monomer) (Figure 8). Subgroup analysis showed that compound CHM could significantly improve the sucrose preference test results ($P < 0.001$), with moderate heterogeneity ($\chi^2 = 35.23$; $I^2 = 55$; df = 16; $P = 0.004$). The effect of monomer CHM was similar to that of the control ($P = 0.06$), with considerable heterogeneity ($\chi^2 = 121.88$; $I^2 = 96$; df = 5; $P < 0.001$). However, there were no statistical differences between subgroups ($P = 0.85$; Figure 8A), with no heterogeneity ($I^2 = 0$). For the open-field test, compound and monomer CHM were both associated with increased activity compared to the control group, but monomer CHM was better than compound CHM; this result was statistically significant ($P = 0.02$, Figure 8B), with considerable heterogeneity ($I^2 = 82.9$). For the forced swimming test, tail suspension test, body weight, and neurological deficit score, the effects of both compound and monomer CHM were better than those of the control group. Still, there was no significant correlation between the form of CHM and effect size ($P = 0.96$, $P = 0.49$, $P = 0.35$, $P = 0.09$, respectively; Figures 8C–F). However, the forest plots showed that monomer CHM improved the neurological deficit scores slightly better than compound CHM. Thus, the different forms of CHM may not be the main source of high heterogeneity.

Publication bias

The Egger's test and funnel plots were performed to assess the potential publication bias. Funnel plots of all the outcomes are shown in Figure 9. The Egger's regression test indicated significant publication bias in relation to the sucrose preference, open-field, and forced swimming tests ($P = 0.015$, $P = 0.014$, and $P < 0.001$, respectively; Figures 10A–C), whereas no obvious risk of publication bias was found for body weight ($P = 0.962$; Figure 10D). Few studies assessed the performed neurological deficit scores and performed the tail suspension test; therefore, we did not conduct a publication bias analysis for them.

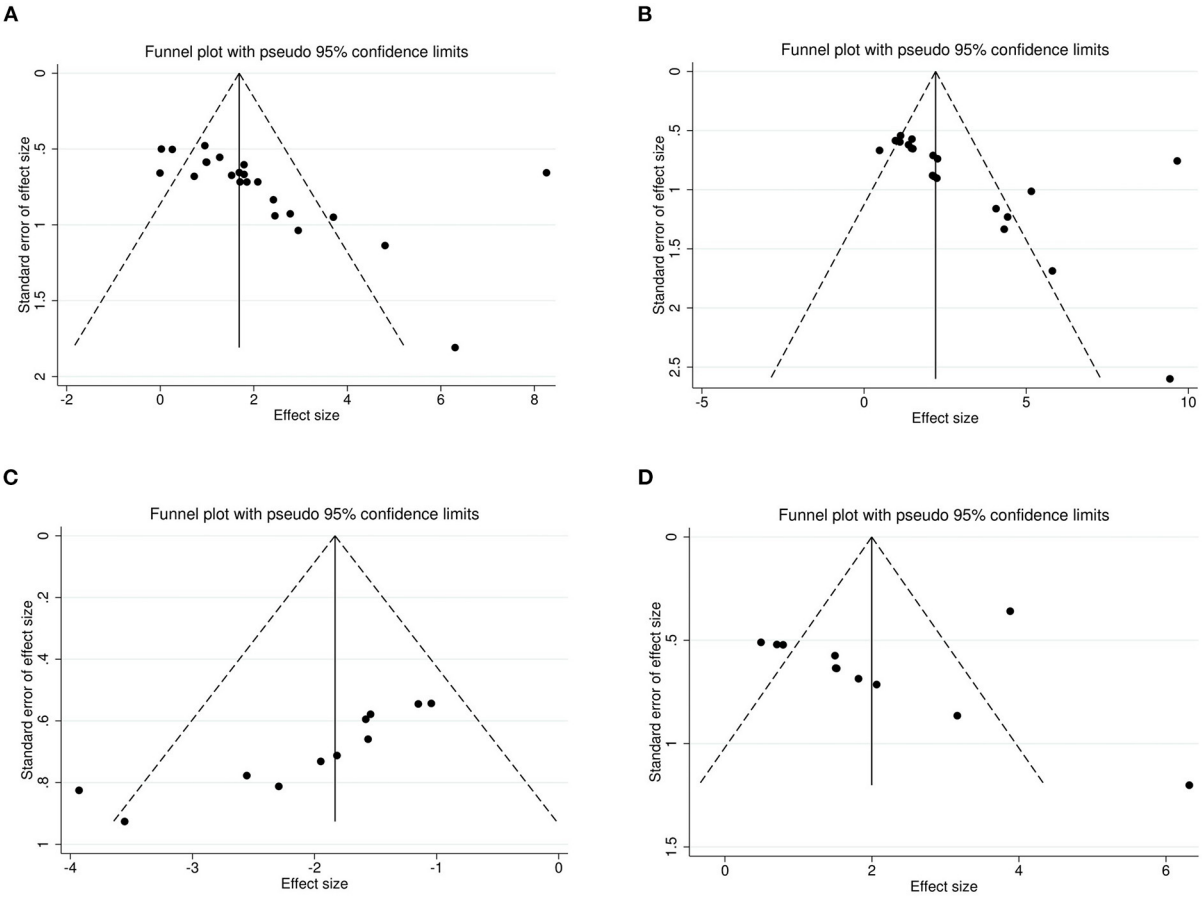


FIGURE 9
Funnel plots for the sucrose preference test **(A)**, open-field test **(B)**, forced swimming test **(C)**, and body weight **(D)** showing the publication bias.

Sensitivity analysis

A sensitivity analysis was performed to assess whether any individual study significantly affected the overall meta-analysis results by removing each study one by one (Supplementary Figure S1). All effect sizes fell within the 95% CI of the overall meta-analysis, indicating that no individual study had an excessive influence on the overall results of the meta-analysis. These results show that our statistical results are relatively stable and reliable.

Discussion

Poststroke depression, the most common psychiatric disorder following a stroke, seriously affects stroke survivors' quality of life and poststroke recovery and also increases their mortality risk (8). The pathogenesis of PSD is not fully understood and is presumably multifactorial. It is thought to involve a combination of various ischemia-induced neurobiological dysfunctions in the context of psychosocial distress (3). The complexity of PSD mechanisms makes prevention and treatment a difficult task. Some western drugs, especially SSRIs, have been proven to be effective. Evidence supports their multi-mechanisms of function. SSRIs are anti-inflammatory agents and enhance neurogenesis through the upregulation of neurotrophins, possibly supported by the stimulation of mitochondrial energy metabolism (3). However, their long-term use is limited due to adverse effects such as bleeding and intracerebral hemorrhage (15). Therefore, it is necessary to explore more effective drugs. Animal studies have found that many CHMs can improve neurological function and depression-like behaviors by regulating neurotrophic factors, hormones, and corresponding signaling pathways and promoting hippocampal neuron regeneration. In this study, we analyzed the effects of CHM on neurological function and depression-like behavior in PSD animals. The pooled results showed that compared with the control group, CHM significantly increased sucrose consumption in the sucrose preference test, increased movement in the open-field test,

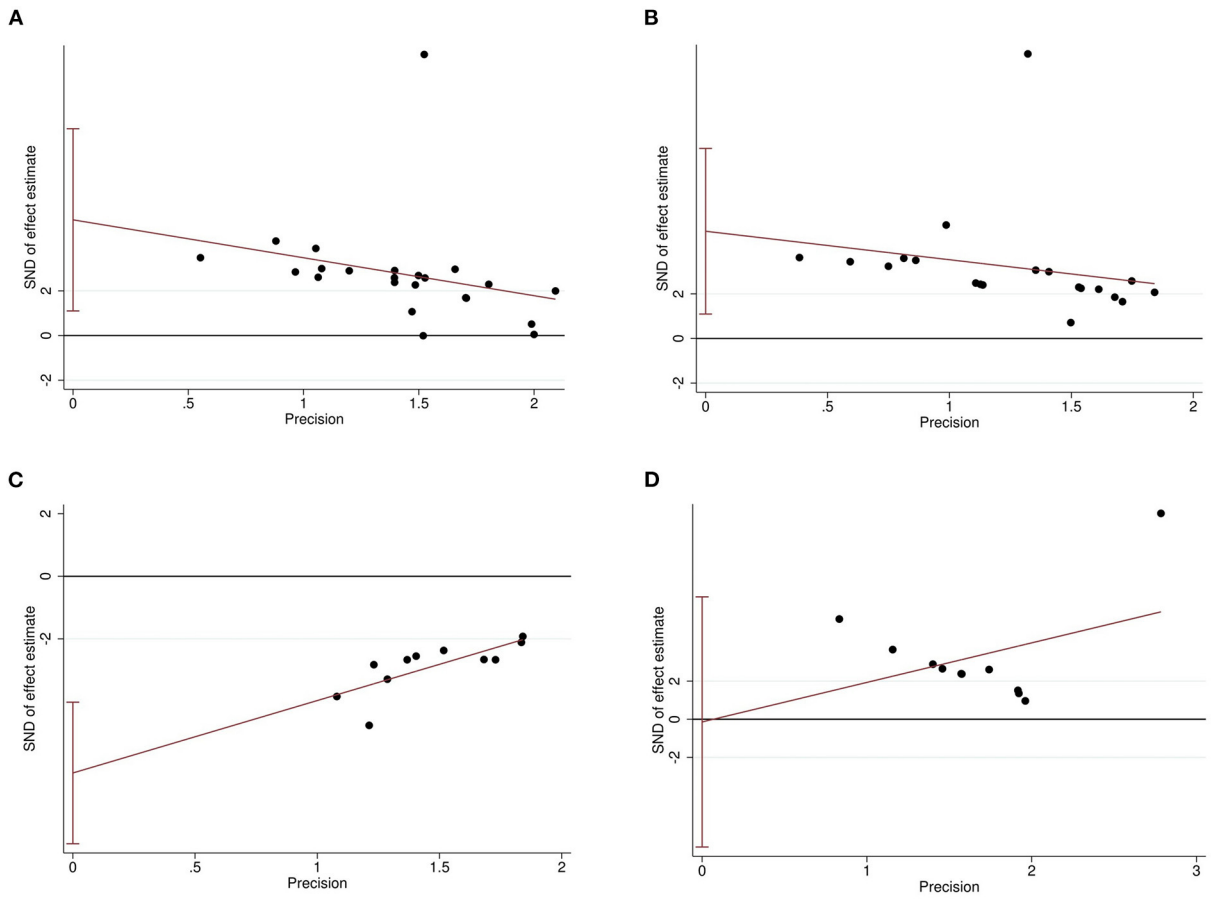


FIGURE 10
Egger's regression for sucrose preference test **(A)**, open-field test **(B)**, forced swimming test **(C)**, and body weight **(D)** confirming potential evidence for publication bias. The vertical lines represent the 95% confidence interval.

increased body weight, and significantly reduced immobility time in the forced swimming and tail suspension tests. These results suggest that CHM can significantly improve PSD behavior. In addition, CHM significantly reduced the neurological deficit score, indicating that the neurological function was improved by CHM.

The subgroup analysis according to the type of CHM showed that neurological deficit score, body weight, and sucrose preference test were statistically different among subgroups. Further analysis showed that each drug was effective in terms of the neurological deficit score. The difference among subgroups may be due to the more obvious effect of peoniflorin and SSA. For body weight, the difference among subgroups may have been due to the more obvious effect of peoniflorin, SSA, and the ineffectiveness of MOOs. For the sucrose preference test, the difference among subgroups may be due to the more obvious effect of peoniflorin and the ineffectiveness of MOOs and SSA. The form of CHM was also analyzed as a subgroup, which showed that monomer CHM was better than compound CHM in terms of the open-field test and neurological deficit score. The former was statistically significant, which could be related to the prominent effect of peoniflorin and SSA. We also found that monomers were not effective in terms of the sucrose preference test due to the negative results of MOOs.

The subgroup analysis showed that monomers were more effective in improving neurological function and some depressive symptoms. Peoniflorin was more effective in improving all six indicators, especially the neurological deficit score, body weight, and sucrose preference test results, which were consistent with the results of the previous systematic analysis (37). In the analysis of the improvement effect of SSA on the sucrose preference test, the *P*-value was considered the critical value. Considering the measurement data error and the significant improvement of SSA on the other five indicators, especially the neurological deficit score and body weight, SSA can still be considered a potential drug for treating PSD. This study found that MOOs had no significant improvement in body weight and the sucrose preference test results with no

significant heterogeneity. Still, MOOs significantly improved the other five indicators, implying that they may improve depressive symptoms other than anhedonia. We also found that HupA improved the neurological deficit score and sucrose consumption and decreased the immobility time in the forced swimming test. However, due to the small sample size and the small number of studies on monomers, this result should be considered with caution. More studies are needed to confirm this finding. For compound preparations, both the overall and subgroup analysis by drug type or form showed that the results of compound CHM were relatively stable and effective. This could be because there are more components in compounds and, thus, more corresponding targets. Our analysis presents new ideas for the clinical treatment of PSD.

There was heterogeneity in the results of some indicators. Unfortunately, due to the relatively small sample size and the lack of information provided in some studies, we did not conduct more subgroup analyses and did not identify the source of heterogeneity. Indeed, Egger's test results and funnel plots suggest that publication bias exists in the current study. The sensitivity analysis suggested that the results were stable and reliable, but our findings should be interpreted with caution due to the small number of studies included.

This study has several limitations. First, we only included studies published in English, which may have led to publication bias. Second, only a limited number of studies were included, and the total sample size was small. Third, additional subgroup analyses could not be performed because of limited data. Fourth, we failed to get all the original data, and some data were measured using ImageJ. Therefore, some errors may have been introduced that could affect the accuracy of the results. Finally, the current studies only investigated the efficacy of CHM on PSD, and the safety of CHM has not been analyzed. In the future, more studies are needed to confirm the safety and efficacy of CHM in the treatment of PSD.

Conclusion

Our results showed that CHM could significantly improve the depression-like behaviors and neurological function of animals with PSD. CHM could be potentially used in the treatment of PSD. However, this study is a meta-analysis based on animal studies. More high-quality preclinical trials and clinical studies are needed to verify our results before CHM can be used in clinical settings.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LL and BH: conception and design, analysis and interpretation of data, drafting the manuscript, and statistical analysis. YuW, TL, and GS: searching the literature. LL and YG: screening of titles and abstracts and full-text data extraction. YaW, YZ, and TZ: critically revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1095444/full#supplementary-material

References

1. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. *Int J Stroke.* (2022) 17:18–29. doi: 10.1177/17474930211065917

2. Medeiros GC, Roy D, Kontos N, Beach SR. Post-stroke depression: a 2020 updated review. *Gen Hosp Psychiatry.* (2020) 66:70–80. doi: 10.1016/j.genhosppsych.2020.06.011

3. Post-stroke depression: Mechanisms and pharmacological treatment. *Pharmacol Ther.* (2018) 184:131–44. doi: 10.1016/j.pharmthera.2017.11.005

4. Castilla-Guerra L, Fernandez Moreno MDC, Esparrago-Llorca G, Colmenero-Camacho MA. Pharmacological management of post-stroke depression. *Expert Rev Neurother.* (2020) 20:157–66. doi: 10.1080/14737175.2020.1707666

5. Gainotti G AA, Marra C. Frequency phenomenology and anatomical-clinical correlates of major post-stroke depression. *Br J Psychiatry*. (1999) 175:163–7. doi: 10.1192/bjp.175.2.163

6. Lipsey SW Jr. Rabins Pv, Robinson Rg. Phenomenological comparison of poststroke depression and functional depression. *Am J Psychiatry*. (1986) 143:527–9. doi: 10.1176/ajp.143.4.527

7. Ezema CI, Akusoba PC, Nweke MC, Uchewoke CU, Agono J, Usoro G. Influence of post-stroke depression on functional independence in activities of daily living. *Ethiop J Health Sci.* (2019) 29:841–6. doi: 10.4314/ejhs.v29i1.5

8. Cai W, Mueller C, Li YJ, Shen WD, Stewart R. Post stroke depression and risk of stroke recurrence and mortality: a systematic review and meta-analysis. *Ageing Res Rev*. (2019) 50:102–9. doi: 10.1016/j.arr.2019.01.013

9. Meng M, Hu G, Yang K, Wang H, Han Y, Pan T, et al. Tuina plus acupuncture for post-stroke depression: a protocol for systematic review and meta-analysis. *Medicine*. (2021) 100:e26013. doi: 10.1097/MD.0000000000026013

10. Guo J, Wang J, Sun W, Liu X. The advances of post-stroke depression: 2021 update. *J Neurol.* (2022) 269:1236–49. doi: 10.1007/s00415-021-10597-4

11. Rachid F BG. Safety and efficacy of repetitive transcranial magnetic stimulation in the treatment of depression: a critical appraisal of the last 10 years. *Neurophysiol Clin.* (2006) 36:157–83. doi: 10.1016/j.neucli.2006.08.006

12. Ayerbe L, Ayis S, Crichton SL, Rudd AG, Wolfe CD. Explanatory factors for the increased mortality of stroke patients with depression. *Neurology*. (2014) 83:2007–201. doi: 10.1212/WNL.0000000000001029

13. Coupland C, Dhiman P, Morriss R, Arthur A, Barton G, Hippisley-Cox J. Antidepressant use and risk of adverse outcomes in older people: population based cohort study. *BMJ*. (2011) 343:15. doi: 10.1136/bmj.d4551

14. Paolucci S. Epidemiology and treatment of post-stroke depression. *Neuropsychiatr Dis Treat.* (2008) 4:145–54. doi: 10.2147/NDT.S2017

15. Legg LA, Rudberg AS, Hua X, Wu S, Hackett ML, Tilney R, et al. Selective serotonin reuptake inhibitors (SSRIs) for stroke recovery. *Cochrane Database Syst Rev*. (2021) 2021:CD009286. doi: 10.1002/14651858.CD009286.pub4

16. Ye Y, Zhu YT, Xin XY, Zhang JC, Zhang HL, Li D. Efficacy of Chinese herbal medicine for tPA thrombolysis in experimental stroke: a systematic review and meta-analysis. *Phytomedicine*. (2022) 100:154072. doi: 10.1016/j.phymed.2022.154072

17. Zhu J, Peng Q, Xu Y, Xu H, Wan Y, Li Z, et al. Morinda officinalis oligosaccharides ameliorate depressive-like behaviors in poststroke rats through upregulating GLUT3 to improve synaptic activity. *FASEB J.* (2020) 34:13376–95. doi: 10.1096/fj.201902546RR

18. Li T, Wang D, Zhao B, Yan Y. Xingnao Jieyu decoction ameliorates poststroke depression through the BDNF/ERK/CREB pathway in rats. *Evid Based Complement Alternat Med.* (2018) 2018:5403045. doi: 10.1155/2018/5403045

19. Li Z, Xu H, Xu Y, Lu G, Peng Q, Chen J, et al. Morinda officinalis oligosaccharides alleviate depressive-like behaviors in post-stroke rats *via* suppressing NLRP3 inflammasome to inhibit hippocampal inflammation. *CNS Neurosci Ther.* (2021) 27:1570–86. doi: 10.1111/cns.13732

20. Wang D, Li T, Han X, He W, Yan Y. Xingnao Jieyu decoction reduces neuroinflammation through the NF-B pathway to improve poststroke depression. *Evid Based Complement Alternat Med.* (2021) 2021:8629714. doi: 10.1155/2021/8629714

21. Wang AR, Mi LF, Zhang ZL, Hu MZ, Zhao ZY, Liu B, et al. Saikosaponin A improved depression-like behavior and inhibited hippocampal neuronal apoptosis after cerebral ischemia through p-CREB/BDNF pathway. *Behav Brain Res.* (2021) 403:113138. doi: 10.1016/j.bbr.2021.113138

22. Page MJ, Mckenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* (2021) 372:n71. doi: 10.1136/bmj.n71

23. Mccann SK, Irvine C, Mead GE, Sena ES, Currie GL, Egan KE, et al. Efficacy of antidepressants in animal models of ischemic stroke: a systematic review and meta-analysis. *Stroke.* (2014) 45:3055–63. doi: 10.1161/STROKEAHA.114.006304

24. Pedder H, Vesterinen HM, Macleod MR, Wardlaw JM. Systematic review and meta-analysis of interventions tested in animal models of lacunar stroke. *Stroke.* (2014) 45:563–70. doi: 10.1161/STROKEAHA.113.003128

25. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* (2002) 21:1539–58. doi: 10.1002/sim.1186

26. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629

27. Du Y, Ruan J, Zhang L, Fu F. Jieyu anshen granule, a chinese herbal formulation, exerts effects on poststroke depression in rats. *Evid Based Complement Alternat Med.* (2020) 2020:7469068. doi: 10.1155/2020/7469068

28. Fan W, Wang Q, Yan Y. Influence of Xingnao Jieyu capsule on hippocampal and frontal lobe neuronal growth in a rat model of post-stroke depression. *Neural Regen Res.* (2012) 7:187–90. doi: 10.3969/j.issn.1673-5374.2012.03.005

29. Tian H, Li X, Tang Q, Zhang W, Li Q, Sun X, et al. Yi-nao-jie-yu prescription exerts a positive effect on neurogenesis by regulating notch signals in the hippocampus of post-stroke depression rats. *Front Psychiatr.* (2018) 9:483. doi: 10.3389/fpsyt.2018.00483

30. Yan Y, Fan W, Liu L, Yang R, Yang W. The effects of Xingnao Jieyu capsules on post-stroke depression are similar to those of fluoxetine. *Neural Regen Res.* (2013) 8:1765–72. doi: 10.3969/j.issn.1673-5374.2013.19.004

31. Zhao A, Ma B, Xu L, Yao M, Zhang Y, Xue B, et al. Jiedu tongluo granules ameliorates post-stroke depression rat model *via* regulating NMDAR/BDNF signaling pathway. *Front Pharmacol.* (2021) 12:662003. doi: 10.3389/fphar.2021.662003

32. Zhao Z, Zhang W, Zhang Y, Zhang Y, Zheng C, Tian H, et al. Multimodal magnetic resonance imaging and therapeutic intervention with yi-nao-jie-yu decoction in a rat model of post-stroke depression. *Front Psychiatr.* (2020) 11:557423. doi: 10.3389/fpsyt.2020.557423

33. Wang HY, Chen M, Zhang BH. Effect of different therapies of Chinese medicine on the expressions of c-Fos and c-Jun proteins in hippocampus of rats with post-stroke depression. *Neural Regen Res.* (2006) 1:234–8.

34. Yan Y, Li T, Wang D, Zhao B, Zhou Q. Antidepressant effect of Xingnao Jieyu decoction mediated by alleviating neuroinflammation in a rat model of post-stroke depression. *J Tradit Chin Med.* (2019) 39:658–66.

35. Hu MZ, Wang AR, Zhao ZY, Chen XY, Li YB, Liu B. Antidepressant-like effects of paeoniflorin on post-stroke depression in a rat model. *Neurol Res.* (2019) 41:446–55. doi: 10.1080/01616412.2019.1576361

36. Du Y, Liang H, Zhang L, Fu F. Administration of Huperzine A exerts antidepressant-like activity in a rat model of post-stroke depression. *Pharmacol Biochem Behav.* (2017) 158:32–8. doi: 10.1016/j.pbb.2017.06.002

37. Wang XL, Wang YT, Guo ZY, Zhang NN, Wang YY, Hu D, et al. Efficacy of paeoniflorin on models of depression: a systematic review and meta-analysis of rodent studies. *J Ethnopharmacol.* (2022) 290:115067. doi: 10.1016/j.jep.2022.115067

frontiers | Frontiers in Neurology

TYPE Systematic Review
PUBLISHED 10 January 2023
DOI 10.3389/fneur.2022.1056156

OPEN ACCESS

EDITED BY
Jingling Chang,
Beijing University of Chinese Medicine, China

REVIEWED BY
Jacopo Lanzone,
Sant'Isidoro Hospital Ferb Onlus Trescore Balneario, Italy
Carmen Parra-Farinas,
University of Toronto, Canada

*CORRESPONDENCE
LiHua Gu
✉ kmglh169@163.com

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 28 September 2022
ACCEPTED 13 December 2022
PUBLISHED 10 January 2023

CITATION
Feng S, Tang M, Huang G, Wang J, He S, Liu D and Gu L (2023) EMG biofeedback combined with rehabilitation training may be the best physical therapy for improving upper limb motor function and relieving pain in patients with the post-stroke shoulder-hand syndrome: A Bayesian network meta-analysis.
Front. Neurol. 13:1056156.
doi: 10.3389/fneur.2022.1056156

EMG biofeedback combined with rehabilitation training may be the best physical therapy for improving upper limb motor function and relieving pain in patients with the post-stroke shoulder-hand syndrome: A Bayesian network meta-analysis

Sisi Feng[1], Mingzhi Tang[1], Gan Huang[1], JuMei Wang[1], Sijin He[2], Duo Liu[2] and LiHua Gu[2]*

[1]Yunnan University of Traditional Chinese Medicine, Kunming, China, [2]Department of Rehabilitation, Kunming Hospital of Traditional Chinese Medicine, The Third Affiliated Hospital of Yunnan University of Chinese Medicine, Kunming, China

Background: Post-stroke shoulder-hand syndrome (SHS), although not a life-threatening condition, may be the most distressing and disabling problem for stroke survivors. Thus, it is essential to identify effective treatment strategies. Physical therapy is used as a first-line option for treating SHS; however, it is unclear which treatment option is preferred, which creates confusion in guiding clinical practice. Our study aims to guide clinical treatment by identifying the most effective physical therapy interventions for improving clinical symptoms in patients with post-stroke SHS using Bayesian network meta-analysis.

Methods: We conducted a systematic and comprehensive search of data from randomized controlled trials using physical therapy in patients with SHS from database inception to 1 July 2022. Fugl-Meyer Upper Extremity Motor Function Scale (FMA-UE) and pain visual analog score (VAS) were used as primary and secondary outcome indicators. R (version 4.1.3) and STATA (version 16.0) software were used to analyze the data.

Results: A total of 45 RCTs with 3,379 subjects were included, and the intervention efficacy of 7 physical factor therapies (PFT) combined with rehabilitation training (RT) was explored. Compared with the control group, all the PFT + RT included were of statistical benefit in improving limb motor function and pain relief. Also, our study indicated that EMG biofeedback combined with RT (BFT + RT) [the surface under the cumulative ranking curve (SUCRA) = 96.8%] might be the best choice for patients with post-stroke SHS.

Conclusion: EMG biofeedback combined with rehabilitation training may be the best physical therapy for improving upper limb motor function and relieving

pain in patients with post-stroke SHS according to our Bayesian network meta-analysis results. However, the above conclusions need further analysis and validation by more high-quality RCTs.

Systematic review registration: www.crd.york.ac.uk/prospero/, identifier: CRD42022348743.

KEYWORDS

stroke, shoulder-hand syndrome (SHS), physical therapy, rehabilitation training, network meta analyses

Introduction

Shoulder-hand syndrome (SHS), also known as reflex sympathetic dystrophy (RSD), is mainly characterized by local pain, limitation of upper extremity movement with swelling, abnormal skin temperature, and skin changes. As a common complication in stroke patients with hemiplegia, usually occurring in patients within 1–3 months after stroke, SHS is a crucial factor affecting the recovery of motor function in the upper extremity of patients (1, 2). Nonetheless, failure to provide timely and unreasonable interventions may prolong SHS patients' recovery, even resulting in permanent deformities of the shoulder, upper limb, and finger, which may seriously affect their daily lives and prognoses (3).

Modern medicine has not yet elucidated the pathogenesis of SHS after stroke. It may be related to reflex sympathetic nerve damage that leads to a series of inflammatory and autoimmune reactions, and the generation of abnormal cytokines (4). Furthermore, limb paralysis impairs the circulation of body fluids in the upper limb of patients with stroke, leading to stasis edema in the affected limb and shoulder-hand pump dysfunction. This may be an essential reason for the pathogenesis of SHS (5). Various microtraumas, such as repeated blood draws, intravenous injections, or inappropriate active and passive motion, might also contribute to or exacerbate SHS (6, 7).

The current clinical treatment of post-stroke SHS focuses on reducing pain while maintaining and restoring function for patients. Drug treatment mainly includes oral anti-inflammatory, analgesic, immune modulating (such as glucocorticoids and non-steroidal anti-inflammatory drugs) and anticonvulsant and antidepressant drugs or injection of stellate nerve block, steroid hormone joint cavity injection closure, intravenous bisphosphonate injection, intradermal injection of botulinum toxin, and other invasive drugs (8–11). While pharmacological treatment is convenient and quick, its long-term use will produce side effects such as infection, poor compliance, and drug resistance. Consequently, it can only relieve some clinical symptoms but cannot fundamentally control and treat the occurrence and development of SHS (12). The treatment guidelines (13) highlight that since pain and limb dysfunction are the main clinical problems associated with SHS, early physical therapy intervention is the basis and first-line choice for SHS treatment. In addition, most experts, even those who use more invasive interventional techniques, agree that effective treatment should emphasize functionally focused interventions, particularly physical therapy that aims at normalizing the function of the affected limb and alleviating problems associated with disuse (14).

Physical therapy, as the main body of rehabilitation treatment, includes exercise therapy based on rehabilitation training and physical factor therapy (PFT) with various physical factors (sound, light, cold, heat, point, magnetic, and water) as the primary means. Although exercise therapy is an indispensable intervention to SHS treatment, some patients still refuse to use the affected limb because of severe pain or experience huge emotional stress. It makes it difficult for them to stick to the treatment and thus reduces its expected efficacy (15, 16). PFT (as a safe and effective alternative therapy) not only provides anti-inflammatory, analgesic, neuromuscular excitation, and spasticity relief *via* the mediating impact of electrotherapeutic stimulation but is also easily accepted by patients due to the comfort of the treatment procedure (17). Various PFT techniques are often combined with rehabilitation training (RT) in clinical practice to treat SHS, and its efficacy is good. However, the advantages of different PFT vary, and there are no relevant guidelines to rank their efficacy on patients with SHS, which confuses the clinical guiding practice. Therefore, we aim to conduct a comprehensive review of RCTs of different physical factor therapies combined with rehabilitation training for the treatment of post-stroke SHS using Bayesian network meta-analysis (NMA), expecting to find the optimal physiotherapy regimen to guide clinical practice.

Abbreviations: SHS, shoulder-hand syndrome; RSD, reflex sympathetic dystrophy; RCTs, randomized controlled trials; NMA, network meta-analysis; PFT, physical factor treatment; FMA-UE, Fugl-Meyer upper extremity motor function scale; VAS, visual analog score of pain; RT, rehabilitation training; ET, electrotherapy; LT, light therapy; UWT, ultrasonic wave therapy; CHT, conductive heat therapy; PT, pressure therapy; MT, magnetic therapy; BFT, biofeedback therapy; EMG-BF, electromyographic biofeedback.

Materials and methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extended statement (18). This NMA has been registered on the International prospective register systematic reviews (PROSPERO) with the registration number CRD42022348743. No ethical approval or patient consent was required for this study since all analyses were conducted based on previously published studies.

Search strategy

We conducted a comprehensive search of the following databases: Web of Science, PubMed, EMBASE, Cochrane Central Controlled Trials, China Knowledge Network (CNKI), Wanfang database, VIP database, and China Biomedical Literature Database (CBM). With no restrictions on language or publication time, we identified the randomized controlled trials (RCTs) on the observation of the efficacy of physiotherapy on post-stroke SHS published before 1 July 2022.

By combining medical subject headings (MeSH) with free words using Boolean logic operators, we integrated the following terms for a comprehensive search: "stroke," "cerebral infarction," "cerebral hemorrhage," "shoulder-hand syndrome," "reflex sympathetic dystrophy," "complex localized pain syndrome type I," "electrotherapy," "low-frequency pulsed electrical stimulation," "neuromuscular electrical stimulation," "transcutaneous electrical nerve stimulation," "ultrasound," "ultrashort wave," "infrared therapy," "laser therapy," "wax therapy," "wet-hot compress," "air wave pneumatic therapy," "hyperbaric oxygen," "magnetotherapy," "transcranial magnetic stimulation," "biofeedback therapy," "electromyographic biofeedback therapy," "rehabilitation training," and "randomized controlled trial." Moreover, we manually screened the reference lists in the relevant meta-analyses and reviews to minimize the omission of literature that meets the inclusion criteria. Taking the PubMed search as an example, details of the search strategy are shown in Supplementary Table 1. Two independent authors (SSF and MZT) processed the screening records using Endnote 20 literature management software (Thompson ISI Research Soft, Philadelphia, Pennsylvania, USA). Disagreements in this process were resolved by discussion or by a third author (LHG).

Selection and exclusion criteria

The inclusion of studies meeting the criteria should be based on the PICOS framework:

Population: Patients were diagnosed with post-stroke SHS according to clear diagnostic criteria (19, 20), without restriction to gender or age.

Intervention: Acceptable treatment is mainly various physical factor therapy (PFT) combined with rehabilitation training (RT). PFT includes electrotherapy (ET), light therapy (LT), ultrasound therapy (UWT), conductive heat therapy (CHT), and pressure therapy (PT). As well as magnetotherapy (MT), which is based on transcranial magnetic stimulation, and biofeedback therapy (BFT), which is based on electromyography biofeedback (EMGBF) as the main intervention. However, there are no restrictions on the frequency, duration, and waveform of the above PFT.

Among them, ET contains low-frequency pulsed electrical stimulation, transcutaneous neuromuscular electrical stimulation, medium-frequency electrotherapy, and ultrashort wave; LT, infrared radiation and laser therapy; UWT, ultrasound and extracorporeal shock wave; CHT, Chinese herbal wet and hot compresses and wax therapy; PT, air pressure, air wave pressure therapy, and hyperbaric oxygen.

Comparison: RT alone or intercomparison between interventions.

Outcomes: Primary outcomes: Fugl-Meyer Upper Extremity Motor Function Scale (FMA-UE). Secondary outcomes: Visual analog score of pain (VAS).

Study design: Randomized controlled trials only. Non-randomized controlled studies, such as animal trials, reviews, systematic reviews, case-control studies, and study protocols, were excluded.

Based on the criteria set above, two authors (GH and JMW) independently screened the titles and abstracts to exclude duplicates and studies that did not meet the inclusion criteria. Subsequently, the eligible studies were reviewed in full. Any inconsistencies that arose during this period were decided by consensus.

Data extraction and quality assessment

Following the Cochrane Consumer and Communications Review Group's data extraction template, we completed relevant data collection for eligible studies: including basic publication information (first author's name and year of publication), participant characteristics (total sample size, age, and duration of disease), interventions, duration of treatment, and quality of RCTs, among other relevant information.

The quality of each eligible study was assessed by two independent investigators (MZT and GH) using the Cochrane Risk of Bias Tool (21). A total of seven areas were covered (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessments, incomplete data on outcome data, selective reporting, and other biases). Each item was rated as unknown, low, or high risk of bias. The assessment was performed in Review Manager (version 5.4).

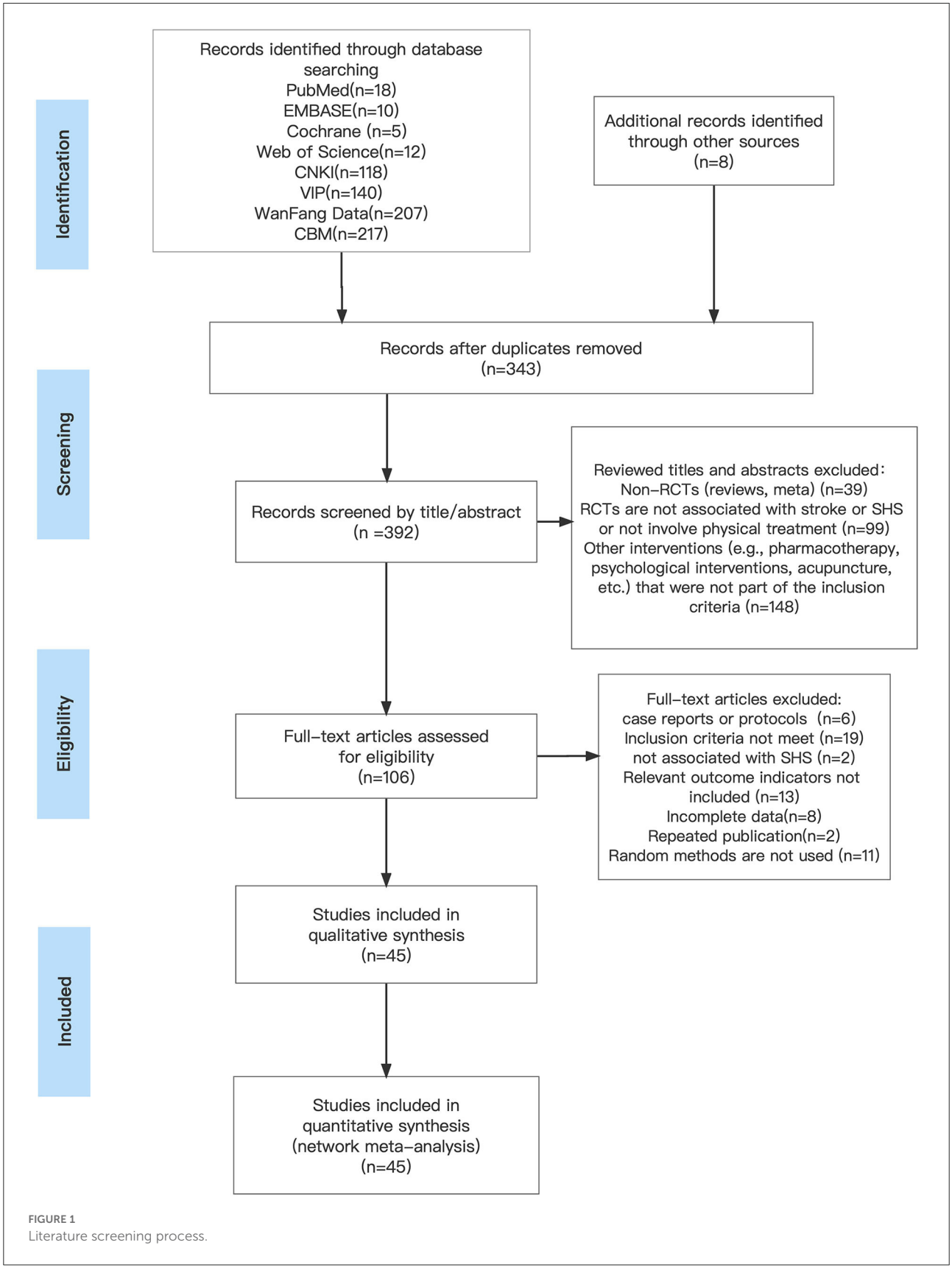


FIGURE 1
Literature screening process.

Statistical analyses

According to the minimally informative prior distributions of the Bayesian random effects model (22), we first performed a conventional pair-wise meta-analysis by synthesizing the essential data from all the included studies. Evaluated effect sizes for each pair-wise treatment comparison in terms of continuous outcome, mean difference (MD) was calculated along with 95% credible intervals (CrIs) as the pooled relative effect and estimate uncertainly, respectively. As a visual representation of statistical heterogeneity, I^2 statistic was tested to assess whether substantial heterogeneity existed. The values 25, 50, and 75% indicated mild, moderate, and high heterogeneity, respectively (18). To detect whether any bias was generated, a comparison-adjusted funnel plot was made as a concise description, and both were analyzed using the Egger test (23). We constructed a network plot for offering all the existing relationships, with distinct treatments expressed by different nodes and trials by lines joining appropriate nodes.

Network transitivity is the most crucial assumption underlying NMA, whose assessment would affect our further analysis directly (24). Therefore, to ensure the sufficient similarity of various treatment comparisons, which can provide valid indirect inferences, we evaluate the transitivity assumption by comparing the clinical and methodological characteristics, such as the characteristics of participants and experimental design, across all the included studies (25, 26). In order to simulate an accurate estimation of the statistical model, four parallel Markov chains were first established in the random selection state (27). Each chain generated 50,000 iterations. Due to the burn-in period, an initial 20,000 iterations were discarded to minimize the bias of initial values when the chain reached its target distribution (28). The Brooks-Gelman-Rubin diagnostic was used to evaluate the convergence of the models by visually inspecting the historical trajectory of trace combined with density plots (29) (see Supplementary Figure 1 for details). As the estimated probability of ranking the physical treatments, the surface under the cumulative ranking curve (SUCRA) was presented as a simple numerical summary statistic cumulative ranking probability plot for each treatment (30). SUCRA with a higher value denotes a greater likelihood of a given treatment being in the top rank or highly effective. In contrast, the value "zero" indicates that the treatment is sure to be the worst. Finally, to explore whether potential source inconsistency arises in our network, we use the "node splitting" technique, comparing direct and indirect evidence across the network (when $P > 0.05$ indicates that consistency arises) (31, 32). The above analyses were performed using the "Gemtc" package (version 1.0–1) and "rjags" (version 4–13) in R software (version 4.1.3), and STATA (version 16.0) software (StataCorp, College Station, TX, USA).

Results

Search process and baseline characteristics

We initially retrieved 735 literature studies, of which 343 were duplicates. After the screening of titles and abstracts, 286 documents were excluded. We reviewed the remaining 106 studies for full text; 6 studies were presented as case reports or study protocols; 2 studies were diagnosed with other types of disease; 11 studies did not adopt the method of random grouping; 19 studies did not meet the inclusion criteria for this study; 13 studies did not provide relevant outcome indicators for our analysis; 8 studies were not available in full text or had incomplete outcome indicators, and another 2 were duplicate published studies. Thus, 45 clinical randomized controlled trials that meet the inclusion criteria were finally included (33–78).

Figure 1 depicts the processing of the literature screening.

Table 1 summarizes key characteristics such as participant baseline information and interventions in detail. The included studies were from China, and the literature was published between 2008 and 2022. A total of 3,379 study participants were randomly assigned to either the trial or control group. Of these, 1,696 participants were included in the trial group of seven different physical factor therapies combined with rehabilitation training (BFT + RT, $n = 135$; CHT + RT, $n = 352$; PT + RT, $n = 259$; ET + RT, $n = 379$; MT + RT, $n = 132$; UWT + RT, $n = 174$; LT + RT, $n = 265$). The remaining 1,683 individuals were randomized into four control groups (CHT + RT, $n = 69$; ET + RT, $n = 60$; LT + RT, $n = 81$; RT, $n = 1,473$).

Quality of included studies

Summary tables of individual and overall level quality assessments are detailed in Supplementary Figures 2, 3. All 45 studies (33–78) reported group randomization, but allocation concealment was unclear. Due to intervention limitations, only two studies (33, 40) adopted the single-blind method for participants; four studies (40, 59, 71, 75) evaluated the study results using the blind method. Five studies (36, 41, 45, 63, 65) reported detailed cause shedding. All 45 included studies reported on the pre-specified outcomes completely. In addition, two studies (44, 60) mentioned no adverse effects.

Network analysis results

Primary outcome: FMA-UE

The preliminary conventional meta-analysis observed a high degree of heterogeneity in the FMA-UE score among studies ($I^2 = 88.2\%$, $P = 0.000$). The adjusted funnel plots showed

TABLE 1 Characteristics of included studies.

Study ID	Participant		Age	Gender (M/F)	Interventions		Course	Outcome
	T	C			T	C		
Zhang et al. (79)	100	100	T: 57.10 ± 1 0.88 C: 56.30 ± 10.72	T: 57/43 C: 59/41	CHT + RT	RT	30d	FMA-UE VAS
Zhao et al. (80)	51	50	T: 58.12 ± 2.41 C: 57.89 ± 2.37	T: 27/24 C: 27/23	LT + RT	RT	28d	FMA-UE
Tian et al. (81)	28	29	T: 65.90 ± 9.50 C: 66.97 ± 10.51	T: 15/13 C: 14/15	MT + RT	RT	2w	FMA-UE VAS
Wu (33)	28	28	T: 59.4 ± 10.7 C: 58.5 ± 9.5	T: 15/13 C: 15/13	MT + RT	RT	27d	FMA-UE VAS
Li et al. (34)	25	25	T: 62.28 ± 13.79 C: 61.68 ± 11.91	T: 22/3 C: 20/5	UWT + RT	RT	4w	FMA-UE VAS
Liu and Wang (35) (a)	50	50	T: 60.2 ± 10.8 C: 62.7 ± 10.7	T: 29/21 C: 27/23	MT + RT	RT	2w	FMA-UE VAS
Ren et al. (40)	40	40	T: 51.64 ± 7.47 C: 57.28 ± 10.66	T: 22/18 C: 24/16	CHT + RT	RT	4w	FMA-UE VAS
Chen and Zheng (43)	27	27	T/C: 63.8 ± 8.4	/	BFT + RT	CHT + RT	4w	FMA-UE VAS
Li and Lai (42)	40	40	T: 62.2 ± 8.9 C: 61.7 ± 9.3	T: 23/17 C: 24/16	ET + RT	RT	4w	FMA-UE VAS
Zhang et al. (41) (a)	30	30	T: 60.1 ± 7.31 C: 59.1 ± 7.9	T: 18/12 C: 19/11	ET + RT	RT	4w	FMA-UE VAS
Weng et al. (46)	30	30	T: 68.82 ± 3.34 C: 68.85 ± 3.36	T: 18/12 C: 19/ 11	CHT + RT	RT	4w	FMA-UE VAS
Li (50)	15	15	T: 48.7 ± 5.3 C: 46.5 ± 6.8	T: 10/5 C: 8/7	PT + RT	RT	21d	VAS
Wu et al. (51)	30	30	T: 54.5 ± 6.5 C: 56.2 ± 7.6	T: 18/12 C: 16/14	PT + RT	RT	18d	FMA-UE
Cai et al. (55)	37	33	T: 57.14 ± 3.99 C: 58.36 ± 4.48	T: 17/20 C: 16/17	ET + RT	RT	47d	FMA-UE VAS
Lin et al. (56)	42	42	T: 63.1 ± 8.3 C: 62.5 ± 9.2	T: 24/18 C: 25/17	BFT + RT	CHT + RT	4w	FMA-UE VAS
Li et al. (57) (a)	30	30	T: 64.7 ± 16.9 C: 65.4 ± 17.3	T: 19/11 C: 17/13	UWT + RT	ET + RT	4w	FMA-UE VAS
Hu et al. (65)	36	36	T: 61.2 ± 17.8 C: 60.1 ± 18.2	T: 20/16 C: 21/15	PT + RT	RT	4w	FMA-UE VAS
She et al. (64)	30	30	T: 57.39 ± 3.18 C: 59.13 ± 4.53	T: 18/12 C: 16/14	ET + RT	RT	4w	FMA-UE VAS
Zhao and Ma (66)	25	25	T: 63.3 ± 4.6 C: 60.2 ± 5.8	T: 18/7 C: 16/9	LT + RT	RT	20d	FMA-UE

(Continued)

TABLE 1 (Continued)

Study ID	Participant		Age	Gender (M/F)	Interventions		Course	Outcome
	T	C			T	C		
Zhang et al. (67)	35	33	T: 61.4 ± 10.9 C: 60.8 ± 11.3	T: 20/15 C: 20/13	ET + RT	RT	3w	VAS
Liu and Dong (71)	20	20	T: 63.7 ± 11.4 C: 62.8 ± 12.1	T: 14/6 C: 13/7	ET + RT	RT	3w	FMA-UE VAS
Su and Chen (72)	30	30	T: 61 C: 63	T: 19/11 C: 18/12	PT + RT	RT	30d	FMA-UE VAS
Yang et al. (36)	31	31	T: 71.81 ± 9.95 C: 72.42 ± 9.68	/	ET + RT	RT	4w	VAS
Liu et al. (37) (b)	40	39	T: 63.38 ± 9.22 C: 64.21 ± 9.35	/	ET + RT	RT	2w	FMA-UE VAS
Qiao and Ding (39)	51	51	T: 53.45 ± 5.48 C: 53.56 ± 5.34	T: 22/29 C: 23/28	CHT + RT	LT + RT	28d	FMA-UE VAS
Gong et al. (45)	30	30	/	/	CHT + RT	LT + RT	21d	FMA-UE VAS
Guo and Ruan (62)	60	60	T: 63.1 ± 3.2 C: 61.1 ± 2.6	T: 36/24 C: 37/23	CHT + RT	RT	3w	VAS
Zhou et al. (49)	20	20	T: 63.71 ± 6.45 C: 63.12 ± 6.89	T: 16/4 C: 15/5	ET + RT	RT	6w	FMA-UE VAS
Yuan and Chen (59)	40	40	T: 51.73 ± 11.16 C: 51.66 ± 11.01	T: 24/16 C: 22/18	PT + RT	RT	10d	FMA-UE VAS
Shi et al. (61)	40	40	T: 52.73 ± 11.17 C: 52.65 ± 10.03	T: 24/16 C: 22/18	ET + RT	RT	4w	FMA-UE VAS
Guo and Ruan (62)	36	31	T: 52 C: 48	T: 25/12 C: 19/12	BFT + RT	RT	4w	FMA-UE VAS
Wang et al. (63)	40	40	T: 65.8 ± 12.6 C: 66.3 ± 12.6	T: 27/13 C: 23/17	LT + RT	RT	4w	FMA-UE VAS
Liu et al. (68)	46	46	T: 62.4 ± 9.6 C: 61.4 ± 10.2	T: 29/17 C: 28/19	PT + RT	RT	4w	FMA-UE VAS
Zhang and Huang (70)	45	45	T: 52.63 ± 9.67 C: 51.26 ± 10.13	T: 25/20 C: 26/19	PT + RT	RT	15d	FMA-UE VAS
Yang et al. (50)	56	56	T: 56.85 ± 10.7 C: 56.72 ± 10.12	T: 31/25 C: 29/27	ET + RT	RT	14d	FMA-UE
Tan (48)	41	41	T: 56.56 ± 3.34 C: 56.23 ± 3.16	T: 23/18 C: 24/17	CHT + RT	RT	10d	FMA-UE VAS
Bao et al. (38)	30	30	T: 63.32 ± 6.13 C: 64.82 ± 8.27	T: 16/14 C: 16/14	UWT + RT	RT	4w	FMA-UE VAS
Zhang et al. (44) (b)	29	29	T: 53.91 ± 5.33 C: 53.70 ± 5.73	T: 13/16 C: 15/14	UWT + RT	RT	4w	FMA-UE VAS

(Continued)

TABLE 1 (Continued)

Study ID	Participant		Age	Gender (M/F)	Interventions		Course	Outcome
	T	C			T	C		
Zhang and Huang (47)	26	26	T: 51.31 ± 7.32 C: 53.18 ± 9.40	T: 16/10 C: 17/9	MT + RT	RT	4w	FMA-UE VAS
Liu et al. (74)	32	31	T: 58.84 ± 6.12 C: 60.04 ± 5.95	T: 17/15 C: 18/13	PT + RT	RT	30d	FMA-UE VAS
Wang et al. (60)	54	54	T/C: 55.27 ± 13.5	/	LT + RT	RT	4w	FMA-UE
Xue et al. (54)	30	30	T: 62.7 ± 5.4 C: 63.4 ± 6.7	T: 15/15 C: 14/16	BFT + RT	RT	6w	FMA-UE
Yan et al. (82)	30	30	T: 53.52 ± 15.32 C: 53.85 ± 15.13	T: 16/14 C: 17/13	UWT + RT	RT	4w	FMA-UE
Lu et al. (58)	80	80	T: 62.2 ± 4.9 C: 63.4 ± 4.9	T: 46/34 C: 44/36	LT + RT	RT	4w	FMA-UE VAS
Li et al. (57) (b)	30	30	T: 64.7 ± 16.9 C: 65.4 ± 17.3	T: 19/11 C: 17/13	UWT + RT	ET + RT	2w	FMA-UE VAS

ET, electrotherapy; LT, light therapy; UWT, ultrasonic wave therapy; CHT, conduction heat therapy; PT, pressure therapy; MT, magnetic therapy; BFT, biofeedback therapy; RT, rehabilitation training; FMA-UE, fugl-meyer upper extremity motor function scale; VAS, visual analog score of pain; C, control group; T, treatment group; d, day; w, week.

a relatively symmetrical distribution of studies on both sides of the inverted funnel. However, some smaller studies are distributed below and outside the inverted funnel, suggesting the possible presence of publication bias (Supplementary Figure 1). An additional Egger's test was used for secondary verification of the presence of publication bias, which showed $P = 0.933$ (>0.05), indicating that there is no publication bias in this study (Supplementary Table 2).

We constructed a visual network geometry showing all the main evidence of the interventions. Each node represents one intervention, and its size depends on the number of patients directly studied. As shown in Figure 2, the most common intervention method was ET + RT with nine groups studied ($n = 313$), followed by PT + RT ($n = 259$) involving seven groups, CHT + RT ($n = 292$) and UWT + RT ($n = 174$) involving six groups, and LT + RT ($n = 265$) involving five groups. Two other interventions [BFT + MT ($n = 135$) and MT + RT ($n = 132$)] involved four groups.

In terms of the outcome of FMA-UE, the efficacy of various physical factor therapies (PFT) combined with rehabilitation training (RT) post-intervention is shown in Figure 3. BFT + RT [MD = 10.21 95%CrI (6.85, 13.58)]; CHT + RT [MD = 8.36 95%CrI (5.91, 10.82)]; PT + RT [MD = 7.60 95%CrI (5.41, 9.80)]; UWT + RT [MD = 7.41 95%CrI (4.86, 9.96)]; MT + RT [MD = 6.06 95%CrI (3.09, 9.02)]; ET + RT [MD = 5.98 95%CrI (4.09, 7.88)]; and LT + RT [MD = 4.30 95%CrI (2.00, 6.60)] efficacy were all statistically significant and significantly superior to the control group. BFT + RT [MD = 5.91 95%CrI (2.07, 9.76)]; CHT + RT [MD = 4.06 95%CrI (1.19, 6.93)]; and PT + RT [MD = 3.30 95%CrI (0.13, 6.48)] were all superior to LT + RT. Meanwhile, BFT + RT [MD = 4.23 95%CrI (0.37, 8.09)] also outperformed ET + RT.

We plotted SUCRA lines to rank each intervention category (Figure 3 and Supplementary Figure 5) and compared them with other interventions. BFT+RT (SUCRA = 94.7%) had the highest probability of improving upper extremity motor function in patients with post-stroke SHS, followed by two equally remarkable interventions CHT+RT (SUCRA = 76.0%) and PT+RT (SUCRA = 65.6%), and the fourth-ranked UWT+RT (SUCRA = 62.3%). In contrast, MT+RT (SUCRA = 42.3%), ET+RT (SUCRA = 39.3%), and LT+RT (SUCRA = 19.8%) had relatively low probabilities, while the probability of RT (SUCRA = 0%) was the lowest. The existence of inconsistencies between direct and indirect evidence was assessed by the "nodal split" method. The results (Supplementary Figure 6) showed that there are no significant inconsistencies in each branch of the entire network ($P > 0.05$) [CHT + RT vs. RT ($P = 0.566$); LT + RT vs. RT ($P = 0.123$); UWT + RT vs. RT ($P = 0.496$); ET + RT vs. RT ($P = 0.498$); BFT + RT vs. RT ($P = 0.321$); LT + RT vs. CHT + RT ($P = 0.123$); BFT + RT vs. CHT + RT ($P = 0.325$); and ET + RT vs. UWT + RT ($P = 0.50$)]. Thus, we obtained a valid comparison of the above-mentioned different physical therapy interventions to improve the function of the upper limb of SHS after stroke.

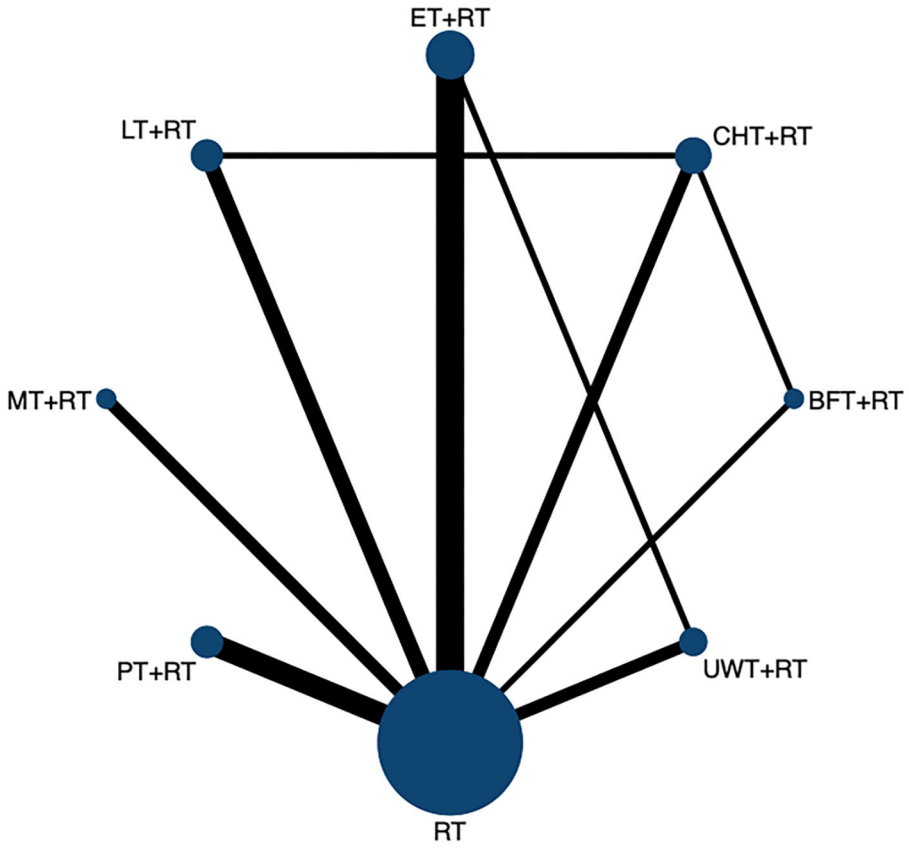


FIGURE 2
The network evidence graph for FMA-UE. RT, rehabilitation training; ET, electrotherapy; LT, light therapy; UWT, ultrasonic wave therapy; CHT, conduction heat therapy; PT, pressure therapy; MT, magnetic therapy; BFT, biofeedback therapy.

Secondary outcome: VAS

The I^2 values indicated that our preliminary meta-analysis showed high heterogeneity in VAS scores across all included studies ($I^2 = 82.2\%$, $P = 0.000$). Comparison-adjusted funnel plot suggested that the occurrence of publication bias depends on several scattered points that are asymmetrically distributed below and outside the inverted funnel plot (Supplementary Figure 7). In addition, Egger's test confirmed this result ($P = 0.011$) (Supplementary Table 3).

The network diagram is shown in Figure 4, including seven interventions and four control groups. ET + RT was the most frequent intervention and investigated in 9 arms ($n = 303$), followed by the most common intervention of CHT + RT ($n = 352$) and PT + RT ($n = 244$) involving 7 arms; UWT + RT ($n = 144$) involving 5 arms; MT + RT ($n = 132$) involving 4 arms; BFT + RT ($n = 105$) involving 3 arms; and LT + RT ($n = 120$) was the least involving only 2 arms.

The clinical efficacy of VAS pain relief results showed (Figure 5) that when compared with the control group, except for LT + RT, the other interventions showed better efficacy: BFT + RT [MD = −2.10 95%CrI (−3.01, −1.20)]; PT + RT [MD = −1.92 95%CrI (−2.53, −1.31)]; CHT + RT [MD = −1.57 95%CrI (−2.12, −1.03)]; ET + RT [MD = −1.33 95%CrI (−1.80, −0.85)]; UWT + RT [MD = −1.28 95%CrI (−1.99, −0.57)]; and MT + RT [MD = −1.94 95%CrI (−1.94, −0.40)]. In addition, BFT + RT [MD = −1.49 95%CrI (−2.59, −0.40)]; PT + RT [MD = −1.31 95%CrI (−2.29, −0.33)]; and CHT + RT [MD = −0.96 95%CrI (−1.73, −0.20)] were also significantly superior than LT + RT.

Plotting the SUCRA line to rank each intervention's efficacy in pain relief (Figure 5 and Supplementary Figure 8) showed that BFT + RT (SUCRA = 89.9%) obtained the best probability compared to the other seven interventions. However, PT + RT (SUCRA = 84.9%) and CHT + RT (SUCRA = 65.8%) also got a remarkable ranking among them, followed by ET + RT (SUCRA = 50.1%); UWT + RT (SUCRA = 48.3%); and MT + RT (SUCRA = 41.9%). LT + RT (SUCRA = 18.3%) and RT (SUCRA = 0.9%) ranked last. The node-splitting model results showed (Supplementary Figure 9) no significant inconsistency between the direct and indirect evidence ($P > 0.05$), so the current evidence is reliable.

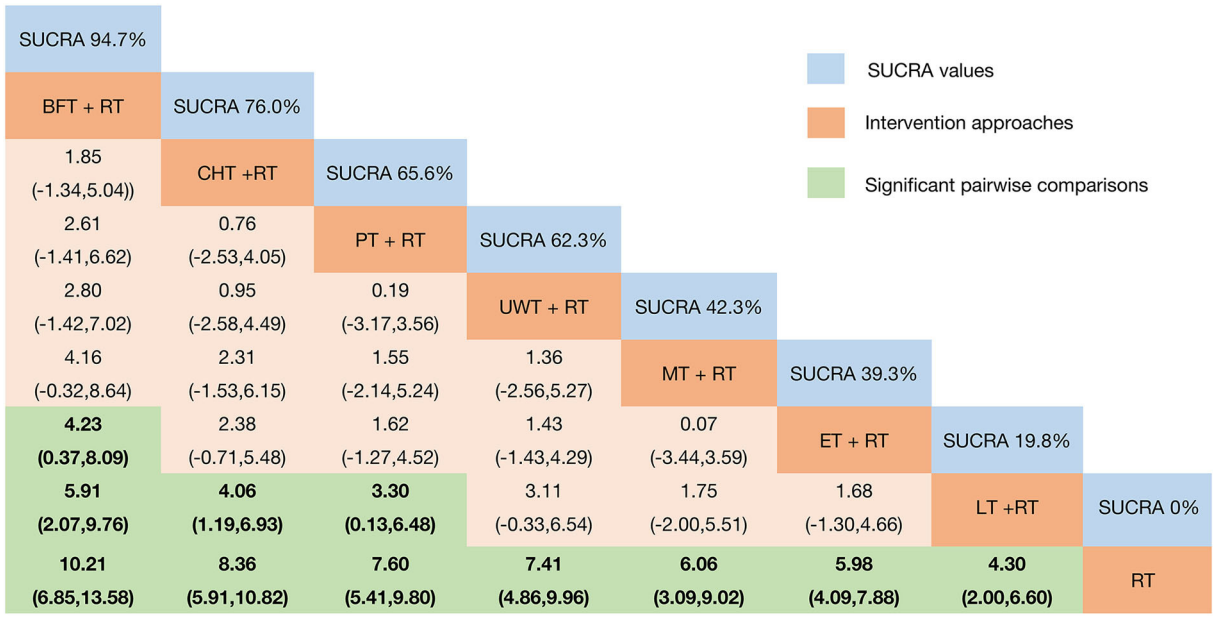


FIGURE 3
Relative effect sizes of FMA-UE efficacy after the intervention according to network meta-analysis. Treatments were ranked in order of their likelihood of being the best treatment. The numbers in the blue boxes are SUCRA values, representing the rank of the treatments. Meaningful pairwise comparisons are highlighted in green and bold. RT, rehabilitation training; ET, electrotherapy; LT, light therapy; UWT, ultrasonic wave therapy; CHT, conduction heat therapy; PT, pressure therapy; MT, magnetic therapy; BFT, biofeedback therapy.

Discussion

Existing RCTs have only analyzed the relative effectiveness of individual physical therapy interventions in terms of their respective efficacy in patients with post-stroke SHS. At the same time, traditional meta-analyses have only been used to assess the effectiveness of a particular intervention. It all lacks comprehensive comparative analyses between studies, but the NMA overcomes this limitation. Network meta-analysis integrates at least two or more physical interventions by performing direct and indirect cross-comparisons with the help of techniques that adjust indirect comparisons while assessing their effectiveness and performing relative ranking on all physical therapy interventions included (75). To the best of our knowledge, this is the first study to use the NMA approach to compare the efficacy of different physical therapy for patients with SHS after stroke. This complex integrated approach is superior to most previous studies, and it can be used as an evidence-based clinical guideline to provide reference evidence for the selection of optimal protocols for the future clinical treatment of SHS.

Post-stroke SHS is a complex disease that threatens the recovery of patients with stroke, and it is essential to identify effective treatment strategies. Although rehabilitation is effective in treating SHS, pain is the primary reason that prevents patients from receiving SHS treatment. In addition, it leads to resistance psychology in some patients, affecting their treatment outcomes (20, 83). Evidence suggests that combining two or more therapies may be more effective than rehabilitation alone in improving the post-stroke SHS symptoms of patients. Physical therapy, in particular, has shown superior performance in reducing pain and improving motor function as the first-line treatment choice for this disease (17, 84–86). Among them, biofeedback therapy (BFT) with EMG biofeedback as the primary intervention combined with rehabilitation training (RT) may offer the potential for the treatment of SHS. In this study, both FMA and VAS results showed that BFT + RT [(MD = 10.21 95%CrI (6.85, 13.58), (SUCRA = 94.7%); (MD = −2.10 95%CrI (−3.01, −1.20), (SUCRA = 89.9%)] is the best treatment strategy to improve upper limb motor function and reduce pain in patients with SHS.

Electromyographic biofeedback (EMG-BF) therapy, a branch of biofeedback therapy (BFT), combines biofeedback techniques with electrical stimulation to promote the reconstruction of undamaged nerve cells and the development of new neural networks after stroke (84). By amplifying the bioelectrical activity of muscle tissue, which the patient is unaware of under normal circumstances, and processing the signal, the signal is fed back to the human body as intuitive visual and auditory signals and further fed back to the brain center. The brain control center regulates muscle contraction and diastole intensity based on the feedback signal and receives

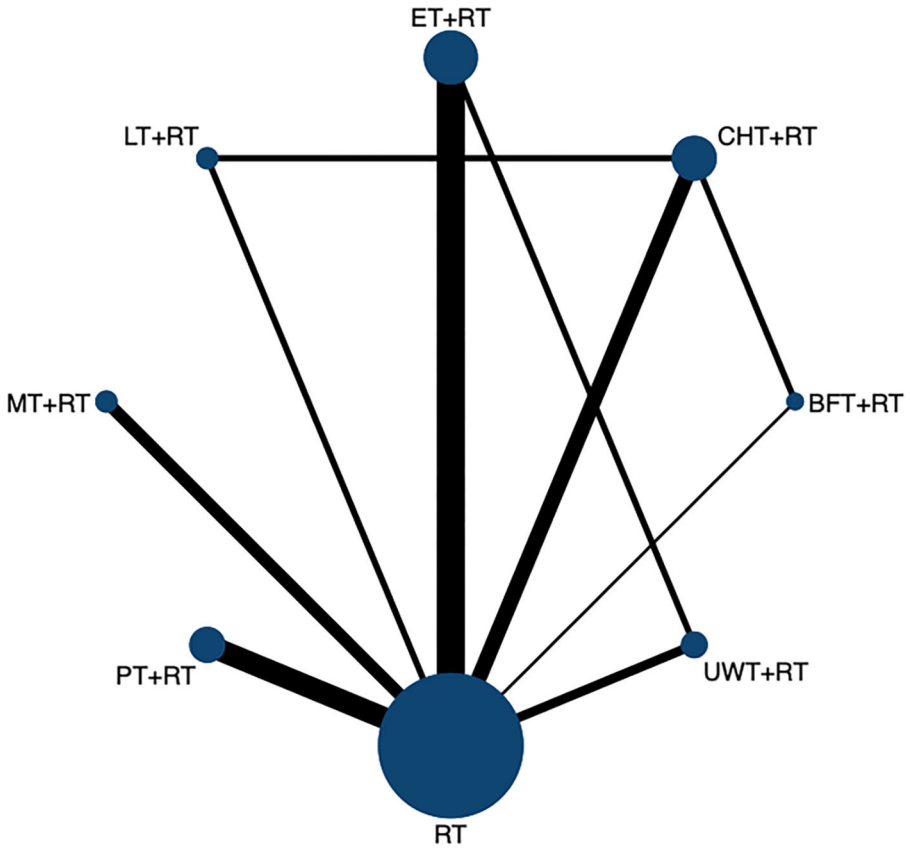


FIGURE 4
The network evidence graph for VAS. RT, rehabilitation training; ET, electrotherapy; LT, light therapy; UWT, ultrasonic wave therapy; CHT, conduction heat therapy; PT, pressure therapy; MT, magnetic therapy; BFT, biofeedback therapy.

active rehabilitation training to achieve the goal of training and treatment (85). According to the results of the meta-analysis of this study, we found that there are statistical differences in the comparison of the efficacy of BFT + RT and electrical stimulation therapy (ET) combined with RT [MD = 4.23 95%CrI (0.37, 8.09)] in improving limb motor function. The results also confirmed the advantages of EMGBF treatment. It overturns the traditional notion that autonomic nerves cannot be controlled arbitrarily and allows patients to dynamically access electromyographic physiological information at the site of information collection, enabling them to learn to consciously regulate their psychophysiological activity to treat somatic disorders (86). It has been demonstrated (87) that EMG-BF provides an additional benefit for the recovery of limb function in patients with stroke when combined with conventional rehabilitation. Moreover, its efficacy is undoubtedly substantial. In addition to promoting the recovery of neurological deficits after stroke, it also helps patients overcome pain-induced resistance to training and motivates them to participate actively in rehabilitation (88, 89). Related studies (43) found that using surface EMG-BF to treat stroke patients with SHS can improve patients' ability to control and regulate random movements significantly. Meanwhile, it also stimulates their desire to train, which transforms passive rehabilitation into active rehabilitation, and improves patients' compliance with training, leading to improved patient outcomes.

In contrast, the potential mechanism of EMG biofeedback in pain relief remains unclear. Related studies found (90) that through the "stimulation-feedback" mode, EMGBF is capable of converting subtle EMG signals into visual stimuli, thereby motivating patients to engage in active exercises of the core muscles of the affected shoulder to stabilize the shoulder joint and alleviate pain. The problem is that when hemiplegic shoulder pain is caused by the interaction of multiple etiologies, a single therapy may not be able to achieve the desired level of pain relief (91, 92). However, our study draws the opposite conclusion, which may be related to our combing EMG biofeedback with rehabilitation training and thus improved efficacy; or it may be associated with the lack of direct evidence between interventions. Speculation on this contradictory view still needs to be validated by more extensive RCTs of the

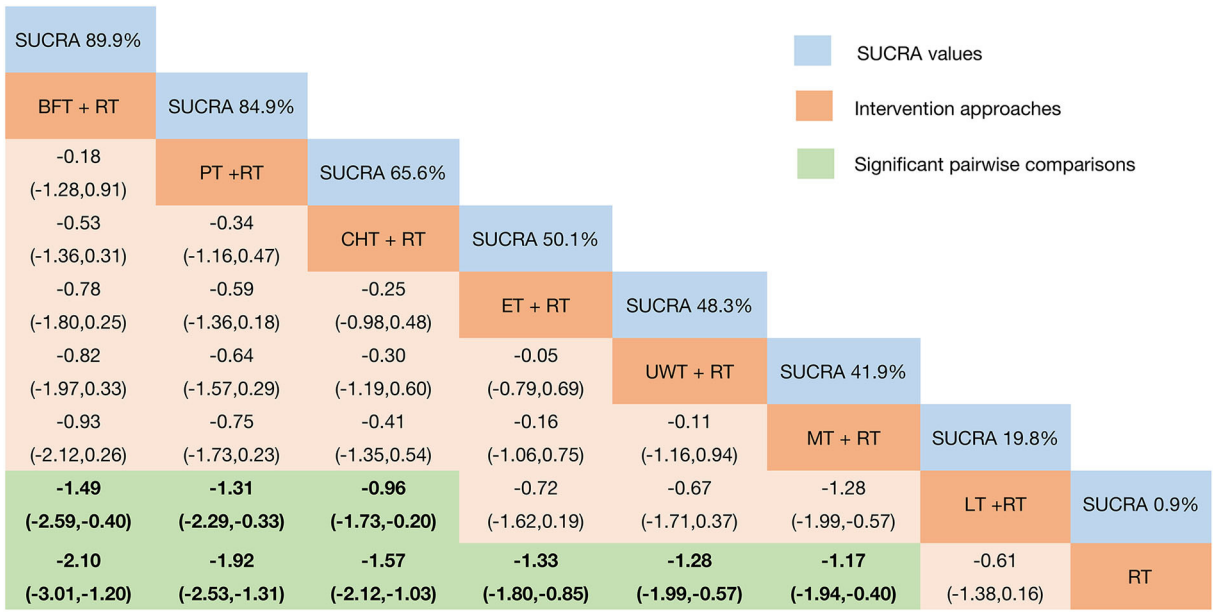


FIGURE 5
Relative effect sizes of VAS efficacy after the intervention according to network meta-analysis. Treatments were ranked in order of their likelihood of being the best treatment. The numbers in the blue boxes are SUCRA values, representing the rank of the treatments. Meaningful pairwise comparisons are highlighted in green and bold. RT, rehabilitation training; ET, electrotherapy; LT, light therapy; UWT, ultrasonic wave therapy; CHT, conduction heat therapy; PT, pressure therapy; MT, magnetic therapy; BFT, biofeedback therapy.

combined treatment with myoelectric biofeedback on shoulder pain in the future.

Also noteworthy is that CHT + RT and PT + RT rank relatively high among all interventions and can be used adjunctively for post-stroke SHS. CHT mainly consists of paraffin wax therapy and moist heat compress therapy. Wax therapy, as a particular conductive medium, uses this principle of warming to conduct heat through the skin to deep tissues, accelerating tissue repair, promoting cellular metabolism, reducing the tension of tissue fibers, and increasing their elasticity. It thereby facilitates muscle strength recovery and enhances joint mobility. At the same time, the warming effect can reduce the excitability of the nerve, improve blood circulation, and finally, reduce inflammatory edema, and accelerate the removal of pain-causing mediators. Furthermore, when the wax is cooled, its fixed condition exerts a local oppressive impact on the body's tissues, aiding in the eradication of swelling and having a better effect on the relaxation of the affected joint ligaments, muscles, and tendons (93). Clinical studies have demonstrated that functional training of the upper extremity soon following the wax therapy can help patients better participate in the training and complete their rehabilitation activities better (46, 94). Wet heat compress therapy, also referred to as Chinese herbal medicine moist heat compress therapy, is often combined with Chinese herbal medicine. Using the combined effects of herbal efficacy and physical thermal effect to select herbs that reduce inflammation, alleviate pain, and relax tendons have many advantages. It can dilate the local blood vessels and open pores, which deepens the drug penetration and gives full play to its effect. Consequently, it improves the time effect of pain symptom relief and facilitated the metabolism of inflammation and edema. Additionally, it significantly increased blood flow to the affected limb's tissues, lowered muscle and ligament tension, and enhanced the flexibility of joints and limb movements, thus improving therapeutic results (95, 96). They have limitations, however, and should be used with caution in patients who have the bleeding tendency in clinical, local sensory abnormality, or wax allergy (86).

Pressure therapy (PT) mainly refers to interstitial pneumatic therapy. With the use of an air pump, the multi-chambered balloon is inflated uniformly and decompressed in an orderly manner, providing centripetal compression from distal to proximal segments of the limb and improving arterial perfusion. It effectively improves arterial blood circulation in the affected limb, thereby eliminating edema and improving peripheral vascular function (66, 97). However, given that pneumatic therapy inflation and deflation are neither based on blood flow blockage and recovery pressure nor does it take into account the influence of the patient's upper limb circumference on the pneumatic therapy pressure, and that patients with stroke frequently have sensory impairment of the affected limb, judging the pneumatic therapy pressure based on the patient's subjective sensation alone lacks scientific validity and may cause

adverse effects (98). Consequently, pneumatic therapy has been used in relatively few RCTs to treat this disease alone, mainly as adjunctive therapy after rehabilitation training to provide muscle relaxation and pain relief. As previously stated, our findings also found that PT + RT is more effective in relieving pain (SUCRA = 84.9%) than improving limb motor function (SUCRA = 65.6%).

In addition, studies showed (99, 100) that ultrasonic wave therapy (UWT) also has mechanical and thermal physical effects. By directly acting on local subcutaneous tissue, the ultrasound emitted from outside the body is concentrated in the deep surface of the tissue and produces a high-energy point, which causes the lesion tissue to absorb energy in a short period of time and rapidly heat up, and produces physical and chemical effects. Ultimately, it promotes local blood circulation, accelerates the absorption of inflammatory factors, reduces the excitability of sensory nerves, and cures pain. However, based on the evidence of this study, UWT did not present a prominent advantage, especially in terms of pain relief (SUCRA = 48.3%). This may be related to the lack of significant differences between various physical therapy interventions and may also be influenced by the number of relevant RCTs available for inclusion, resulting in a lack of more direct comparative evidence. Similarly, the relatively weak ranking of magnetic therapy (MT) with transcranial magnetic stimulation as the main intervention may be explained by the relative paucity of studies on the clinical use of magnetic therapy for SHS compared to others (n = 132). Nevertheless, again, this needs further confirmation.

More noteworthy is that, according to our pooled meta-analysis, no statistical difference was observed in pain relief between the light therapy combined with rehabilitation training (LT + RT) group and the control group. Also, based on the SUCRA values, the top three ranked physiotherapies (BFT + RT, CHT + RT, PT + RT) are all statistical differences compared to LT + RT. Generally, this finding is consistent with recent studies (101), indicating that phototherapy has a weak immediate analgesic effect and that its long-term effectiveness is mainly determined by its ability to repair tissues. Therefore, it is commonly used in the adjunctive treatment of pain diseases. On the contrary, the possible differences in the methodological design of different current studies result from the continuous advancement of medicine and the emergence of new high-energy lasers and helium-neon lasers. However, due to the setting of inclusion criteria and other technical limitations, we failed to explore this aspect in depth. This remains to be analyzed in the future by further collecting more direct evidence.

Limitations

However, our study has some limitations as well. First, our study aims to make comparisons from a macroscopic perspective, thus ignoring the refined specific interventions such as transcutaneous electrical nerve stimulation, intermediate frequency electrotherapy, and hyperbaric oxygen or confounding factors such as different frequencies and different intervention durations. Second, the included studies which were all from China lack ethnic diversity, which may result in the limited generalizability of the findings. Finally, significant differences in sample sizes between physical therapy interventions may also have contributed to imprecise analyses. Compared with the overall sample size (n = 3379), the sample sizes of BFT + RT (n = 135) and MT + RT (n = 132) are relatively small.

Conclusion

Based on the findings of our NMA study, EMG biofeedback therapy combined with rehabilitation training (BFT + RT) is the most effective physiotherapy option for improving upper extremity motor function and relieving pain in patients with the post-stroke SHS, followed by CHT + RT and PT + RT. However, given the macroscopic nature of this study and the lack of direct comparative evidence between multiple countries and centers, future studies need to conduct related randomized controlled trials on more physiotherapy interventions. In addition, it helps to conduct more relevant and refined meta-analyses successfully.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SF conceived the study and wrote the manuscript. MT, GH, and JW participated in the extraction and analysis of the data. The study was critically supervised, evaluated, and validated by LG, SH, and DL. All of the authors worked on the article and agreed with the version that was sent in.

Funding

This study was supported by the National Natural Science Foundation of China (grant number. 81860878) and the Yunnan Provincial Education Department Scientific Research Fund Project (grant number. 2022Y375).

Acknowledgments

The author would like to thank her friends Yiqin Zhong and Ting Zeng for their help in editing and revising this manuscript's images.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.1056156/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Model convergence and density plots.

SUPPLEMENTARY FIGURE 2
Quality assessment percentage graph.

SUPPLEMENTARY FIGURE 3
Summary chart of quality assessment.

SUPPLEMENTARY FIGURE 4
FMA-UE funnel plot.

SUPPLEMENTARY FIGURE 5
Probability ranking results of FMA-UE of different interventions.

SUPPLEMENTARY FIGURE 6
Node-splitting diagram of FMA-UE.

SUPPLEMENTARY FIGURE 7
VAS funnel plot.

SUPPLEMENTARY FIGURE 8
Probability ranking results of VAS of different interventions.

SUPPLEMENTARY FIGURE 9
Node-splitting diagram of VAS.

SUPPLEMENTARY TABLE 1
The search strategy for PubMed.

SUPPLEMENTARY TABLE 2
Egger's test for FMA-UE.

SUPPLEMENTARY TABLE 3
Egger's test for VAS.

References

1. Pertoldi S, Di Benedetto P. Shoulder-hand syndrome after stroke. A complex regional pain syndrome. *Eura Medicophys.* (2005) 41:283–92.

2. Hannan MA, Sabeka MM, Miah MB. Shoulder hand syndrome in hemispheric stroke. *J Neurol Sci.* (2013) 333:e167–e167. doi: 10.1016/j.jns.2013.07.693

3. Hartwig M, Gelbrich G, Griewing B. Functional orthosis in shoulder joint subluxation after ischaemic brain stroke to avoid post-hemiplegic shoulder-hand syndrome: a randomized clinical trial. *Clin Rehabil.* (2012) 26:807–16. doi: 10.1177/0269215511432355

4. Borchers AT, Gershwin ME. Complex regional pain syndrome: a comprehensive and critical review. *Autoimmun Rev.* (2014) 13:242–65. doi: 10.1016/j.autrev.2013.10.006

5. Xiaoyin L, Yingyu Z, Yongxin S. Prevention and treatment of the shoulder-hand syndrome after cerebral stroke for patients by arterio-venous hand pump. *J China Med Univ.* (2012) 41:270–282.

6. Xiangming Y. Research progress on the pathogenesis and comprehensive rehabilitation treatment of shoulder-hand syndrome after stroke. *Pract Geriatr.* (2015) 29:452–6.

7. Kondo I, Hosokawa K, Soma M, Iwata M, Maltais D. Protocol to prevent shoulder-hand syndrome after stroke. *Arch Phys Med Rehabil.* (2001) 82:1619–23. doi: 10.1053/apmr.2001.25975

8. Eisenberg E, Geller R, Brill S. Pharmacotherapy options for complex regional pain syndrome. *Exp Rev Neurother.* (2007) 7:521–31. doi: 10.1586/14737175.7.5.521

9. Lee SK, Yang DS, Lee JW, Choy WS. Four treatment strategies for complex regional pain syndrome type 1. *Orthopedics.* (2012) 35:e834–42. doi: 10.3928/01477447-20120525-21

10. Iolascon G, Moretti A. Pharmacotherapeutic options for complex regional pain syndrome. *Exp Opin Pharmacother.* (2019) 20:1377–86. doi: 10.1080/14656566.2019.1612367

11. Kim YH, Kim SY, Lee YJ, Kim ED. A prospective, randomized cross-over trial of T2 paravertebral block as a sympathetic block in complex regional pain syndrome. *Pain Phys.* (2019) 22:E417–24. doi: 10.36076/ppj/2019.22.E417

12. Zhang XL, Tang CZ, Jia J. Analysis of the current status of Chinese and Western medicine in the treatment of post-stroke shoulder-hand syndrome. *Chin J Rehabil Med.* (2015) 30:294–8.

13. Harden RN, McCabe CS, Goebel A, Massey M, Suvar T, Grieve S, et al. Complex regional pain syndrome: practical diagnostic and treatment guidelines. *Pain Med.* (2022) 23:S1–S53. doi: 10.1093/pm/pnac046

14. Stanton-Hicks MD, Burton AW, Bruehl SP, Carr DB, Harden RN, Hassenbusch SJ, et al. An updated interdisciplinary clinical pathway for CRPS: report of an expert panel. *Pain Pract.* (2002) 2:1–16. doi: 10.1046/j.1533-2500.2002.02009.x

15. Shim H, Rose J, Halle S, Shekane P. Complex regional pain syndrome: a narrative review for the practising clinician. *Br J Anaesth.* (2019) 123:e424–33. doi: 10.1016/j.bja.2019.03.030

16. Wang F, Ye JB, Zhao SR, Zheng CM, Huang CY. The efficacy of comprehensive rehabilitation training combined with intramuscular effect patches on post-stroke shoulder-hand syndrome stage I. *Chin J Rehabil.* (2017) 32:205–6.

17. Smart KM, Wand BM, O'Connell NE. Physiotherapy for pain and disability in adults with complex regional pain syndrome (CRPS) types I and II. *Cochrane Database Syst Rev.* (2016) 2:CD010853. doi: 10.1002/14651858.CD010853.pub2

18. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, et al. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med.* (2015) 162:777–84. doi: 10.7326/M14-2385

19. Chinese Medical Association, Neurology Branch. *2016 Edition of the Chinese Guidelines and Consensus on the Management of Cerebrovascular Disease*. Beijing: People's Health Publishing House (2016), 106–150.

20. Zhu YL, Zhang H, He JJ. *Neurological Rehabilitation*. Beijing: People's Army Medical Publishing House (2010), 791–792.

21. Savović J, Weeks L, Sterne JA, Turner L, Altman DG, Moher D, et al. Evaluation of the Cochrane Collaboration's tool for assessing the risk of bias in randomized trials: focus groups, online survey, proposed recommendations and their implementation. *Syst Rev.* (2014) 3:37. doi: 10.1186/2046-4053-3-37

22. Mengersen K, Stojanovski EJC. *Bayesian Methods in Meta-Analysis 1*. (2006), 116–121. doi: 10.1201/b14674-20

23. Seagroatt V, Stratton I. Bias in meta-analysis detected by a simple, graphical test. *Test had 10% false positive rate BMJ*. (1998) 316:470–1.

24. Salanti G. Indirect and mixed-treatment comparison, network, or multiple-treatments meta-analysis: many names, many benefits, many concerns for the next generation evidence synthesis tool. *Res Synth Methods*. (2012) 3:80–97. doi: 10.1002/jrsm.1037

25. Caldwell DM, Ades AE, Higgins JP. Simultaneous comparison of multiple treatments: combining direct and indirect evidence. *BMJ*. (2005) 331:897–900. doi: 10.1136/bmj.331.7521.897

26. Jansen JP, Naci H. Is network meta-analysis as valid as standard pairwise meta-analysis? It all depends on the distribution of effect modifiers. *BMC Med*. (2013) 11:159. doi: 10.1186/1741-7015-11-159

27. Mavridis D, Salanti G, A. practical introduction to multivariate meta-analysis. *Stat Methods Med Res*. (2013) 22:133–58. doi: 10.1177/0962280211432219

28. Valkenhoef GV, Kuiper J. *gemtc: Network Meta-Analysis Using Bayesian Methods*. John Wiley & Sons Ltd (2015).

29. Brooks S, Gelman A. General methods for monitoring convergence of iterative simulations. *J Comput Graph Stat*. (1998) 7:434–55.

30. Page MJ, Shamseer L, Altman DG, Tetzlaff J, Sampson M, Tricco AC, et al. Epidemiology and reporting characteristics of systematic reviews of biomedical research: a cross-sectional study. *PLoS Med*. (2016) 13:e1002028. doi: 10.1371/journal.pmed.1002028

31. Valkenhoef GV, Dias S, Ades AE, Welton NJ. Automated generation of node-splitting models for assessment of inconsistency in network meta-analysis. *Res Synth Methods*. (2016) 7:80–93. doi: 10.1002/jrsm.1167

32. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z

33. Wu DN. The clinical effects of transcranial magnetic electrotherapy on shoulder hand syndrome after cerebral stroke. *Hebei Med J*. (2021) 43:1044–1050.

34. Li LX, Zhao S, Zhang HD, Shen J. Efficacy of extracorporeal shock wave combined with conventional rehabilitation treatment for post-stroke shoulder-hand syndrome. *Guizhou Med J*. (2021) 45:1555–6.

35. Liu Q, Wang ZF. Observation of clinical effect of rTMS on shoulder and hand syndrome in 50 cases. *Anhui Med Pharm J*. (2021) 25:124–6.

36. Yang LF Li W, Wang SL, Hu Q. Efficacy of neuromuscular electrical stimulation combined with pneumatic compression in the treatment of shoulder-hand syndrome. *Health Wellness Guide*. (2021) 2:51–2.

37. Liu XH, Liu HJ, Guan YM. Effect of low frequency pulse electrical therapy combined with rehabilitation training on patients with shoulder-hand syndrome after stroke. *J Cervicodynia Lumbodynia*. (2021) 42:505–7.

38. Bao J, Shi P, Song S. Clinical effects of subsuspension exercise therapy combined with shock waves in the treatment of post-stroke shoulder-hand syndrome. *Ningxia Med J*. (2021) 43:1015–6.

39. Qiao XX, Ding X. Effect of modified external application of traditional Chinese medicine liquid in patients with stage I post-stroke shoulder-hand syndrome. *Guangxi Med J*. (2020) 42:1463–6.

40. Ren K, Wei JM, Cheng H, Han L. Clinical study on heat ironing of chinese herbal wax combined with routine rehabilitation for shoulder-hand syndrome after stroke. *New Chin Med*. (2020) 52:62–5.

41. Zhang JP Li L, Xu QQ Li F, Wang N. Clinical study of sling exercise training combined with shock wave therapy in treatment of I stage shoulder-hand syndrome after stroke. *Shandong J Tradit Chin Med*. (2019) 38:652–6.

42. Li H, Lai DD. Phase I clinical efficacy of ultrashort wave combined with exercise therapy for hemiplegic patients with shoulder-hand syndrome. *Shenzhen J Integ Tradit Chin West Med*. (2019) 29:144–5.

43. Chen JM, Zheng SY. Clinical study on blood-activating and collaterals-dredging traditional Chinese medicine fumigation combined with surface electromyography biofeedback for post-stroke shoulder syndrome. *Chin Arch Tradit Chin Med*. (2019) 37:2605–8.

44. Zhang C, Liu GY, Xiao SR Yi Z, Wang CL. Observation on effect of TCM directinal medication combined with ultrasound on stage I of shoulder-hand syndrome after stroke. *China Med Pharm*. (2019) 9:51–3.

45. Gong Y, Guo CF, Zhang JY, Wang H. The efficacy of Chinese herbal collapse combined with infrared irradiation on post-stroke shoulder-hand syndrome. *Heilongjiang J Tradit Chin Med*. (2019) 48:4–6.

46. Weng DH, Niu DL, Wang JX. The clinical value of rehabilitation training combined with novel waxing therapy for post-stroke shoulder-hand syndrome. *Lab Med Clin*. (2018) 15:827–30.

47. Zhang GF, Huang HJ. Functional magnetic stimulation in the rehabilitation of post-stroke shoulder-hand syndrome. *Cardiovasc Dis Elect J Integ Tradit Chin West Med*. (2018) 6:80–1.

48. Tan ZY. Clinical efficacy of Chinese herbal hot compresses combined with air wave pressure therapy instrument in the treatment of post-stroke shoulder-hand syndrome. *China Med Device Inform*. (2018) 24:117–8.

49. Zhou KW, Chen XF, Guo X, Chen B. Efficacy of transcutaneous electrical nerve stimulation combined with exercise therapy in the treatment of shoulder-hand syndrome. *Mod Pract Med*. (2017) 29:1012–4.

50. Li J. Efficacy of intermittent pneumatic compression in the treatment of shoulder-hand syndrome in stroke. *Chin J Rehabil*. (2017) 32:210–1.

51. Wu SH, Ye XJ, Gu QY, Chen YS. Clinical effects of pneumatic compression therapy and rehabilitation training in stroke patients with shoulder-hand syndrome stage I. *Heilongjiang Med J*. (2017) 41:222–3.

52. Yang JZ, Zheng FL, Wang ZY Li JW, Xin YC. Analysis of the efficacy of Chinese medicine iontophoresis combined with comprehensive rehabilitation training in the treatment of post-stroke shoulder-hand syndrome. *Neural Injury Funct Reconst*. (2017) 12:358–65.

53. Li JF. *Efficacy of Extracorporeal Shock Wave and Ultrasound in the Treatment of Shoulder-Hand Syndrome*. Jinan: Shandong University (2016).

54. Xue LF, Jiang JF, Zhang WP, Liang KM. Study of early comprehensive rehabilitation care for delayed stroke shoulder-hand syndrome. *Guangxi Med J*. (2016) 38:1036–8.

55. Cai HM, Wang W, Ma D, Wang WJ. The efficacy of ultrashort wave in the stellate ganglion region in patients with shoulder-hand syndrome after cerebral infarction. *Chin J Rehabil Med*. (2016) 31:688–90.

56. Lin FY, Chen DS. The efficacy of Chinese herbal fumigation combined with myoelectric biofeedback therapy in the treatment of post-stroke shoulder-hand syndrome. *Mod J Integ Tradit Chin West Med*. (2016) 25:3377–81.

57. Li JF, Zhang Y, Yue SW. Clinical observation of the extracorporeal shock wave in the treatment of shoulder-hand syndrome. *Chin J Rehabil*. (2016) 31:255–7.

58. Lu HY, Pang QT, Guo GY, Liu P, Song XL. The efficacy of infrared/red light local irradiation combined with comprehensive rehabilitation in the treatment of shoulder-hand syndrome. *Chin J Phys Med Rehabil*. (2015) 37:375–7.

59. Yuan XM, Chen PN. Study on the use of airwave pressure therapy in post-stroke shoulder-hand syndrome. *J Nurses Train*. (2015) 30:1421–3.

60. Wang X, Zhao LB, Xie DX. The observation of effects to treat stroke and shoulder-hand syndrome by laser acupuncture irradiation joint acupoint sticking and rehabilitation training. *Laser J*. (2014) 35:67–8.

61. Shi CX, Xie RJ, Wang XH, Yang YL, Deng Y, Chen L. Researches on randomized controlled trials of patients with shoulder-hand syndrome after stroke by routine rehabilitation training combined with medium-frequency electrotherapy. *Med Res Educ*. (2014) 31:41–5.

62. Guo J, Ruan H. Clinical observation of comprehensive rehabilitation treatment for patients with shoulder-hand syndrome after stroke. *Chin J Trauma Disabil Med*. (2013) 21:291–2.

63. Wang Y, Li BL, Wang AL. He-ne laser is modulated medium frequency electric therapy combined with rehabilitation nursing intervention curative effect observation of shoulder hand syndrome after stroke. *Laser J*. (2013) 34:84–5.

64. She LY, Wang P. The effect of ultrashort wave therapy and movement rehabilitation training on hemiplegic stroke patients with stage I shoulder-hand syndrome. *China Med Herald*. (2013) 10:45–50.

65. Hu KH Li YA, Xiong GH, Cheng K, Qin L, Jiang W, et al. Clinical study of intermittentpressure therapy combined with immersion of hot and cold water alternately on stroke patients with shoulder-hand syndrome. *Chin J Rehabil*. (2013) 28:106–8.

66. Zhao YY, Ma YW. Analysis of the efficacy of pulsed laser combined with intermittent pneumatic compression in the treatment of post-stroke shoulder-hand syndrome. *Chin J Misdiagn*. (2012) 12:1820–1.

67. Zhang XL, Guan QB, Gu XD, Wang DP, Hu YM. Effect of transcutaneous electrical nerve stimulation on sympathetic skin responses in patients with post-stroke shoulder-hand syndrome. *Chin J Phys Med Rehabil*. (2012) 12:920–3.

68. Liu GH, Cui GL, Zhu RX. Air pressure wave treatment for shoulder-hand syndrome after stroke treatment efficacy analysis. *Guide China Med*. (2012) 10:11–3.

69. Guo YH, Chen HX, Yang ZJ Li M. The efficacy of alternating hot and cold herbal bath therapy combined with rehabilitation training in the treatment of post-stroke shoulder-hand syndrome. *Chin J Phys Med Rehabil.* (2011) 04:303–4.

70. Zhang M, Huang Y. Clinical analysis of infrared acupoint irradiation in the treatment of post-stroke shoulder-hand syndrome. *Chin J Pract Nerv Dis.* (2011) 14:60–1.

71. Liu DH, Dong JG. Efficacy of ultrashort wave therapy combined with rehabilitation training in the treatment of post-stroke shoulder-hand syndrome. *Chin J Med.* (2011) 46:58–9.

72. Su Q, Chen QF. Efficacy of intermittent pneumatic compression in the treatment of post-stroke shoulder-hand syndrome. *J Qiqihar Med Univ.* (2010) 31:2539–40.

73. Bi LY, Tang ZY, Qian XH, Ye XL. Clinical observation of microwave irradiation combined with kinesiotherapy for shoulder-hand syndrome. *J Pract Med.* (2008) 3:355–7.

74. Liu M, Huang ZM, Jiang HX. The effect of hyperbaric oxygenation in combination with rehabilitation training in patients with shoulder-hand syndrome after stroke. *Chin J Rehabil Med.* (2008) 2:123–5.

75. Lumley T. Network meta-analysis for indirect treatment comparisons. *Stat Med.* (2002) 21:2313–24. doi: 10.1002/sim.1201

76. Zhang YQ, Ma YH. Progress in the study of scapular dyskinesia in patients with post-stroke shoulder pain. *Chin J Rehabil Med.* (2020) 35:498–501.

77. Green S, Buchbinder R, Hetrick S. Physiotherapy interventions for shoulder pain. *Cochrane Database Syst Rev.* (2003) 2003:CD004258. doi: 10.1002/14651858.CD004016

78. Anandkumar S, Manivasagam M. Multimodal physical therapy management of a 48-year-old female with post-stroke complex regional pain syndrome. *Physiother Theory Pract.* (2014) 30:38–48. doi: 10.3109/09593985.2013.814186

79. Zhang MM, Hao N, Li YL, Guo YN, Xiao WX, Ge JL. Clinical effect of wet compress with Sanggui Shuangzhi Fang combined with wax therapy in the treatment of post-stroke shoulder-hand syndrome. *Jilin J Chin Med.* (2022) 42:566–9.

80. Zhao XK, Zhou JJ, Zhang QQ. Efficacy of high-energy laser in the treatment of early stroke patients with shoulder-hand syndrome. *Acta Med Sin.* (2021) 34:132–6.

81. Tian Y, Geng D, Chen GM Li HB. Clinical effect of high frequency repetitive transcranial magnetic stimulation on shoulder-hand syndrome after stroke. *J Neurosci Mental Health.* (2021) 21:494–8.

82. Yan XY Yu XQ, Yuan MH, Ding H, Zou WB, Wang L, et al. Efficacy observation of occupational therapy combined with ultrasound in the treatment of shoulder and hand syndrome. *Pract Clin J Integ Tradit Chin Western Med.* (2022) 22:109–12.

83. Kraft E, Storz C, Ranker A. Physikalische Therapie in der Behandlung des komplexen regionalen Schmerzsyndroms [Physical therapy in the treatment of complex regional pain syndrome]. *Schmerz.* (2021) 35:363–72. doi: 10.1007/s00482-021-00577-y

84. Gaume A, Vialatte A, Mora-Sánchez A, Ramdani C, Vialatte FB, A. psychoengineering paradigm for the neurocognitive mechanisms of biofeedback and neurofeedback. *Neurosci Biobehav Rev.* (2016) 68:891–910. doi: 10.1016/j.neubiorev.2016.06.012

85. Li JH, Wang J. *Clinical Application of Surface Electromyography Diagnostic* Techniques. Zhejiang: Zhejiang University Press (2015).

86. Yan TB. *Physiotherapy*. Beijing: People's Health Publishing House (2016).

87. Woodford H, Price C, EMG. biofeedback for the recovery of motor function after stroke. *Cochrane Database Syst Rev.* (2007) 2007:CD004585. doi: 10.1002/14651858.CD004585.pub2

88. Tan WB. Design of a surface EMG-based myoelectric biofeedback training and therapy system. *South China Univ Technol.* (2019) 18:8578–86. doi: 10.1109/JSEN.2018.2865623

89. Guo JM. Clinical study on the effect of high frequency repetitive transcranial magnetic stimulation combined with myoelectric biofeedback therapy on the rehabilitation of upper limb motor function in stroke patients with hemiplegia. *Southern Med Univ.* (2019) 20:3754.

90. Wang CE. Effect of combination of acupuncture, massage and electromyographic biofeedback therapy on symptom and shoulder joint function in patients with hemiplegic shoulder pain. *Mod J Integ Tradit Chin West Med.* (2021) 30:2777–81.

91. de Oliveira AKA, da Costa KSA, de Lucena GL, de Oliveira Sousa C, Filho JFM, Brasileiro JS. Comparing exercises with and without electromyographic biofeedback in subacromial pain syndrome: a randomized controlled trial. *Clin Biomech.* (2022) 93:105596. doi: 10.1016/j.clinbiomech.2022.105596

92. Kamonseki DH, Calixtre LB, Barreto RPG, Camargo PR. Effects of electromyographic biofeedback interventions for shoulder pain and function: systematic review and meta-analysis. *Clin Rehabil.* (2021) 35:952–63. doi: 10.1177/0269215521990950

93. Lv FY Li J, Wang LJ. The clinical application of pan-analysis wax therapy. *J Precis Med.* (2006) 6:564.

94. Sun GT, Jiang XX, Wu CJ, Dong SX, Huang ZY. Wax therapy for post-stroke shoulder pain. *Heilongjiang Med Pharmacy.* (2017) 40:52–5.

95. Wang Y. Clinical study on the prevention of local pain caused by peripheral intravenous infusion of 10% potassium chloride solution with Chinese herbal moist heat compresses. *Shanghai Univ Tradit Chin Med.* (2019) 95:100646.

96. Guo JH, Chen LN Li H. Recent research on chinese herbal fumigation therapy. *Lishizhen Med Mater Med Res.* (2000) 10:948–9.

97. MacLellan Donald G, Fletcher John P. Mechanical compression in the prophylaxis of venous thromboembolism. *ANZ J Surg.* (2007) 77:418–23. doi: 10.1111/j.1445-2197.2007.04085.x

98. Zhang DD, Cao F, Zhan Q. Effect of modified air pressure therapy on upper extremity motor function in patients with stage I shoulder-hands syndrome after stroke. *J Neurol Neurorehabil.* (2018) 14:161–6.

99. Liu JY, Qiu L, Zheng X, Zhang M, Zhang J. Clinical application of ultrasound in the treatment of painful diseases. *Chin J Rehabil.* (2013) 28:468–70.

100. Ainsworth R, Dziedzic K, Hiller L, Daniels J, Bruton A, Broadfield J, et al. prospective double blind placebo-controlled randomized trial of ultrasound in the physiotherapy treatment of shoulder pain. *Rheumatology.* (2007) 46:815–20. doi: 10.1093/rheumatology/kel423

101. Liu Y, Fang M, Chen LP, Shen W. Advances in the therapeutic mechanisms and clinical applications of high energy laser therapy in pain disorders. *Chin J Pain Med.* (2020) 26:894–7.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 11 January 2023
DOI 10.3389/fneur.2022.1092127

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Yan Huang,
Shanghai University of Traditional Chinese Medicine, China
Jinsong Liu,
Wuhan Union Hospital, China

*CORRESPONDENCE
Bin Xu
✉ xubin@njucm.edu.cn
Xuan-ming Hu
✉ drhxm@qq.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 07 November 2022
ACCEPTED 21 December 2022
PUBLISHED 11 January 2023

CITATION
Song L-z-x, Li Y, Qian X, Xu N, Yu Z, Dai J-w, Xu B and Hu X-m (2023) Parkinson's disease constipation effect of electroacupuncture at ST25 through colonic motility and enteric neuropathology.
Front. Neurol. 13:1092127.
doi: 10.3389/fneur.2022.1092127

Parkinson's disease constipation effect of electroacupuncture at ST25 through colonic motility and enteric neuropathology

Li-zhe-xiong Song[1,2†], Yuan Li[1,2†], Xu Qian[1,2], Na Xu[1,2], Zhi Yu[1,2], Jing-wen Dai[1], Bin Xu[1,2*] and Xuan-ming Hu[3*]

[1]Key Laboratory of Acupuncture and Medicine Research of Ministry of Education, Nanjing University of Chinese Medicine, Nanjing, China, [2]School of Acupuncture-Moxibustion and Tuina, Nanjing University of Chinese Medicine, Nanjing, China, [3]Acupuncture Department, Nanjing Hospital of Traditional Chinese Medicine, Affiliated to Nanjing University of Traditional Chinese Medicine, Nanjing, China

Background: The enteric nervous system (ENS) plays a central role in developing Parkinson's disease (PD) constipation, and the regulation of the ENS may be a key component in treating PD constipation. Electroacupuncture (EA) can effectively treat constipation symptoms in PD, but research on its specific mechanisms, especially in terms of ENS, is relatively lacking. Therefore, we investigated whether EA at ST25 promotes the restoration of ENS structure and colonic motor function in the rotenone-induced PD constipation rat model.

Methods: In this study, we evaluated constipation symptoms by stool characteristics, excretion and water volume, and whole gut transit time and observed colonic motility regulation through colonic motion detection and pathological changes in the colonic myenteric nervous plexus by transmission electron microscopy and immunofluorescence staining.

Results: EA significantly improved the constipation symptoms and positively adjusted the colonic motility in rotenone-induced PD constipation rats. At the same time, EA reversed the rotenone-induced colonic myenteric nervous plexus injury and regulated the ratio of inhibitory and excitatory neurotransmitters.

Conclusion: Our results indicate that EA treatment of PD constipation may be mediated through the adjustment of ENS.

KEYWORDS

Parkinson's disease constipation, electroacupuncture, enteric nervous system, neuropathology, colonic motility

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by motor and non-motor symptoms (NMS), both of which are associated with increasing age and the course of the disease. Gastrointestinal dysfunction symptoms are one of the most common forms of NMS in PD (1). Among all gastrointestinal symptoms, constipation is the most common manifestation of lower gastrointestinal dysfunction in patients with PD, with a prevalence of 24.6–63% (2). Recent evidence indicates that constipation may also be one of the most common disorders in the prodromal phase of

PD, highlighting its value as a risk factor or predictor of the development of PD (3). The currently available evidence suggests the presence of α-synaptic nuclear protein (α-syn) aggregates and neurotransmitter alterations in intestinal tissue. All these findings support Braak's proposed pathophysiological model of α-syn aggregates in Parkinson's disease, which is the early pathological involvement of the enteric nervous system (ENS) and the dorsal motor nucleus vagus (4, 5). The ENS plays a crucial role in the neurodegenerative process leading to PD (6). The ENS determines the motor patterns of the gastrointestinal tract, processes sensory input from mechanical and chemical receptors in the gut wall, and integrates sympathetic and parasympathetic inputs, interacting with the immune and endocrine systems of the gut to produce coordinated effects (7). All these functions are based on the interaction between the different neuronal subtypes in the ENS, and the balance between the type of input and the level of postsynaptic receptor expression released into the neuronal network by each neurotransmitter (8).

While much progress has been made in understanding the pathogenesis of PD and the symptomatic treatment of PD-related symptoms, there are currently no effective neuroprotective or disease-modifying therapies to slow the progression of the disease. The physical, psychological, social, and economic burden of PD remains the most challenging barrier to treatment, especially in the advanced stages of the disease (9). Treatments for constipation in patients with PD include behavioral changes (e.g., increased water intake and physical activity) and the use of pro-secretory agents or osmotic laxatives. In addition, complementary and alternative therapies combined with TCM such as acupuncture, Tui Na, and Tai Chi are increasingly used in PD (10, 11). In recent years, acupuncture has received increasing attention as a non-invasive treatment method. A growing number of studies have investigated the effectiveness of acupuncture targeting PD and other related disorders, such as motor dysfunction (12–14), anxiety (15), depression (16), insomnia (17), and constipation (18, 19), with some positive results. Although existing systematic evaluations and meta-analyses have shown conflicting results for acupuncture for PD constipation due to significant heterogeneity and small sample sizes (20–24), the fact that acupuncture was considered an effective or safe treatment for functional constipation and gastrointestinal disorders (25, 26) in some randomized trials. Available evidence suggests that acupuncture treatment has the potential to alleviate motor and NMS of PD, but the underlying mechanisms are unclear (27–29). The neuroprotective effects of acupuncture on neurodegenerative lesions in animal models of PD are mainly focused on cerebral neurons (30–32), and no studies have yet reported the effects of acupuncture on the ENS. Here, we investigate the mechanisms of acupuncture to alleviate bowel dysfunction in PD constipation by revealing the effects of acupuncture treatment on the ENS myenteric nervous plexus and its neurotransmitters in an animal model of PD.

Materials and methods

Establishment of the experimental animal model

In this study, 8-week-old Sprague Dawley (SD) rats were supplied by the Beijing Vital River Laboratory Animal Technology Co., Ltd. [No. 110011220101889264, under grant SCXK(JING)2021-0011]. The experimental rats were kept in a barrier environment with stable parameters (conditions: 12/12-h light/dark cycle; temperature, 22 ± 2°C; relative humidity 60 ± 5%). The animals were randomly numbered and divided into three groups: model group, electroacupuncture (EA) group, and control group (for convenience, the following texts refer to them as PD, EA, and SH groups, respectively), with six animals in each group. They were kept in cages of the same size in groups and had free access to food and water. PD was induced by giving a low dose of rotenone. The PD and EA groups were injected subcutaneously with rotenone solvent on the back of the neck at a dose of 0.1 ml/kg once a day for 5 days a week. The solvent was prepared by dissolving 200 mg of rotenone (M6209; Abmole Bioscience Inc, Houston, TX, USA) in 3 ml of dimethyl sulfoxide (DMSO, D8370; Beijing Solarbio Science & Technology, Tongzhou, Beijing, China) and then fixed to 100 ml with sunflower oil to make up 2 mg/ml of rotenone sunflower oil solvent. The SH group was injected with an equal volume of solvent mixture (3% DMSO sunflower oil solvent).

Weekly metabolic cages were performed after rotenone injection to measure dry weight and length of stool (the specific methods of metabolic cage method and stool collection and analysis will be elaborated in Stool Collection and Analysis). The modeled rats were evaluated after 4 weeks of rotenone treatment. Rats with defecation indexes (stool dry weight and length) lower than the SH group mean and PD behavioral score of ≥2 were considered to meet the criteria. The process of the present study is shown in Figure 1. All the experiments were approved by the Scientific Investigation Board of the Nanjing University of Traditional Chinese Medicine, Nanjing , China (permission no. 202112A047) and performed per the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals published by the National Science Council, China (under grant 202006A016).

PD behavioral evaluation

Parkinson's disease behavioral evaluation was performed according to the criteria developed by Chen et al. (33). The

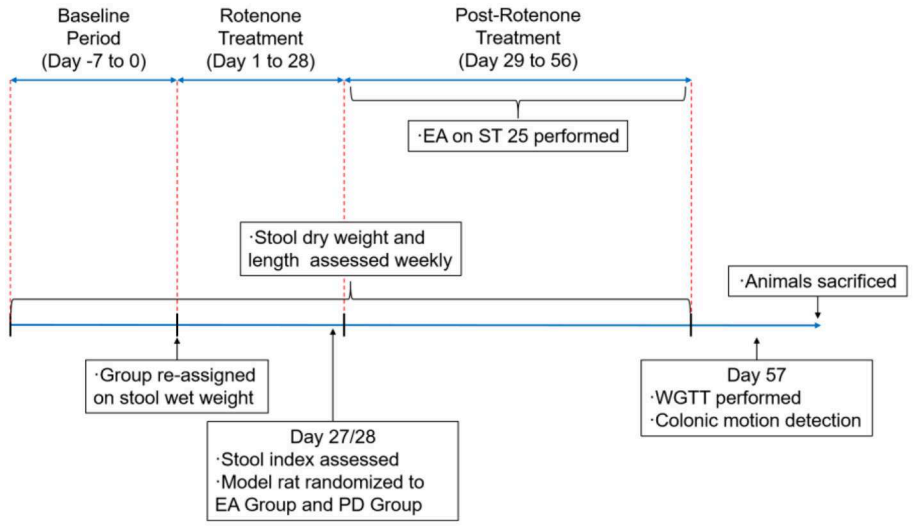


FIGURE 1
Schedule of the experimental procedures. EA, electroacupuncture; PD group, the model group; ST25, acupoint Tianshu; WGTT, whole gut transit time.

TABLE 1 PD behavioral evaluation criteria. The higher score includes the lower scoring performance.

Score	Performance
1	Reduced refusal behavior, yellowing and soiling of the coat, arching of the back, erect hair, and reduced active activity
2	Significantly reduced active movement, slow movement, tremor, or unstable gait
4	Unsteady gait, or the inability to walk in a straight line, or swiveling to one side when walking
6	Reclining to one side, unilateral forelimb and/or hindlimb paralysis, difficulty in walking, difficulty in eating
8	Complete unilateral paralysis of the forelimb and/or hind limb, contracture of the limbs, significant weight loss, inability to eat
10	Dying state or death

specific scoring criteria are shown in Table 1. Only rats with a behavioral score of ≥2 were included in the next step of screening for defecation indicators because rats with a behavioral score of ≥2 have relatively significant neurological deficits and the PD rat models in this range are more reliable (34).

Stool collection and analysis

Rat stools were collected using the metabolic cage method as follows. The rats were placed in individual wire cages with a separation device, which consisted of two layers of separation nets, the upper layer of which separated the rats from the stools to ensure that the stools and the rats' activities will not interact, and the lower layer of which separated the stools from the urine to ensure that the wet weight of the stools will not be affected by the urine. Rats' stools were collected at the end of the first 5 days of treatment each week, and the time was fixed from 7:00 p.m. to 7:00 a.m. for a 12-h period to reduce the error caused by water evaporation.

The stools were collected in sealed bags in the same order as the rats were placed. Then, the stools were dried in a dryer (70°C, 6 h; Septree ST-06; Xinchi e-commerce, Foshan, Guangdong, China) until the weight no longer changed, and the dry weight of the stools was weighed using an analytical balance (QUINTIX313-1CN; Sartorius Scientific Instruments, Shunyi, Beijing, China). The dry–wet difference (ΔW) of the stools is expressed as Wet weight (g)–Dry weight (g). Finally, the stools were arranged by long diameter on a grid paper with a scale to calculate their length.

Whole gut transit time

Whole gut transit time (WGTT) was determined by the interval between the oral gavage of 3 ml of 0.5% phenol red (dissolved in PBS) and the first appearance in stools of red marker (35, 36). The rats after the oral gavage of phenol red were observed visually for the stools and the color of the stools. The actual presence of phenol red in the fecal sample was confirmed by spectrophotometric analysis. As described previously (37), we learned that the WGTT lasted more than 8 h; therefore, starting from the seventh hour after the oral phenol red, the animal cages were checked every 10 min for red in the stools.

Colonic motion detection

The animals were fasted overnight with free access to water and gas anesthesia with isoflurane (2–5%; 9020000522; Shenzhen Ruiwode Lift Technology, Nanshan, Shenzhen, China), and colonic motility was recorded using a previously described method (38). A small balloon made of flexible condom rubber was inserted into the colon 3–6 cm *via* the anus of rats. The pressure in the balloon was measured with a transducer and recorded with a physiological signal-acquisition system (AD Instruments, Pudong, Shanghai, China) for further analysis. Zeroing was performed before and after balloon placement into the animal for 30 min, and *in vitro* zeroing was performed in water. After baseline stabilization, the recording was started. After recording, the balloons were removed and placed in water for re-zeroing to compare with the pre-zeroing data and to prepare for the next animal. After all the animals were finished, the data were processed by LabChart8 software, and the data were randomly selected for three discrete periods of 4 min each. Frequency (Hz), mean (Kpa), min (Kpa), and height (Kpa) were derived using the built-in parameters of the software. The mean colonic pressure formula was Mean (Kpa)–Min (Kpa). The number of peristaltic waves per minute was calculated manually. During the experiment, the temperature of the animal was maintained at 37 ± 0.5°C, using an electric heating board. The experimental procedure is shown in Figure 2.

Transmission electron microscopy

The procedure is described as follows: Quickly fix the tissue with a volume of no more than 1 × 1 × 1 mm in electron microscopy fixation solution at 4°C for 2–4 h without mechanical damage such as traction, contusion, and extrusion and then rinse it three times with 0.1 M phosphate buffer (pH 7.4) for 15 min each. Fix it with 1% osmic acid (0.1 M phosphate buffer, pH 7.4) for 2 h at room temperature (20°C) and then rinse it three times as mentioned earlier. Dehydrate the tissue with gradient alcohol and acetone (50–70–80–90–95–100–100% alcohol-100% acetone-100% acetone for 15 min each time), and then dip it in agent and acetone 812 embedding (acetone: 812 embedding agents = 1:1 for 2–4 h, acetone: 812 embedding agents = 2:1 overnight, pure 812 embedding agents for 5–8 h) for penetration. Insert the sample into the embedding plate full of pure 812 embedding agents and place it in the oven at 37°C overnight and 60°C for 48 h. Slice the sample with an ultra-thin microtome (60–80 nm), stain it with 2% uranyl acetate saturated alcohol solution and lead citrate for 15 min each, and dry them overnight at room temperature. Transmission electron microscopy was used to observe and capture images. Image-pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) software was used for image analysis and data acquisition. One complete plexus was selected for each image, the number of unmyelinated nerves and plexus area (μm^2) was counted, and the nerve density ($/\mu m^2$) was calculated.

Immunofluorescence staining

Frozen sections were used for immunofluorescence (IF) staining. Colon tissue was fixed in 4% paraformaldehyde overnight and dehydrated in 30% sucrose in 0.1 M PBS (Biosharp Life Sciences, China) at 4°C. After embedding in the optimal cutting temperature compound, the colon tissue was sliced into 10-μm thick sections and mounted on slides. The sections were then blocked in 0.2% Triton X-100 (Sigma-Aldrich (Shanghai) Trading Co., Ltd.) for 10 min and permeabilized in Sea BLOCK Blocking Buffer (Thermo Fisher Scientific, USA) for 1 h. They were then incubated with primary antibodies (nNOS, GB11145, 1:2000; ChAT, GB11070-1, 1:500) overnight at 4°C and incubated with secondary antibodies (nNOS, GB23303, 1:500; ChAT, GB25303, 1:400) for 1 h at 37°C. Finally, the tissue sections were covered by coverslips after washing them with 0.1 M PBS. Images were obtained by a fluorescence microscope (Olympus BX60 Darkfield DIC Metallurgical Microscope, Japan).

Electroacupuncture intervention

The rats in the EA group received EA treatment on bilateral ST25 (Tianshu, located 5 mm lateral to the intersection between the upper two-third and the lower one-third in the line joining the xiphoid process and the upper border of the pubic symphysis) after gas anesthesia with isoflurane (2–5%; 9020000522; Shenzhen Ruiwode Lift Technology). Meanwhile, the same anesthesia was administered to rats in the PD group but without performing EA. For the EA group, two stainless steel acupuncture needles (20162270970; Suzhou HUATUO Medical Instruments, Suzhou, Jiangsu, China) of 0.2 mm in diameter were inserted at a depth of 5 mm into the ST25 acupoint. EA at ST25 was conducted with the HANS-100A (HAN ACUTENS WQ1002F; Beijing Anlong Photoelectric Technology, Haidian, Beijing, China) apparatus set to a waveform of the dilatational wave, a current of 2 mA and a frequency of 2/15 Hz, 20 min a day for 5 days a week, 1 week a course, and four continuous courses of treatment.

Data analysis

Data from all the experiments are expressed as mean ± standard error values. Weekly comparison of defecation indicators among groups in Figure 3 using two-way ANOVA. Paired *t*-test was used for comparison before and after rotenone treatment or EA intervention, and an independent *t*-test was

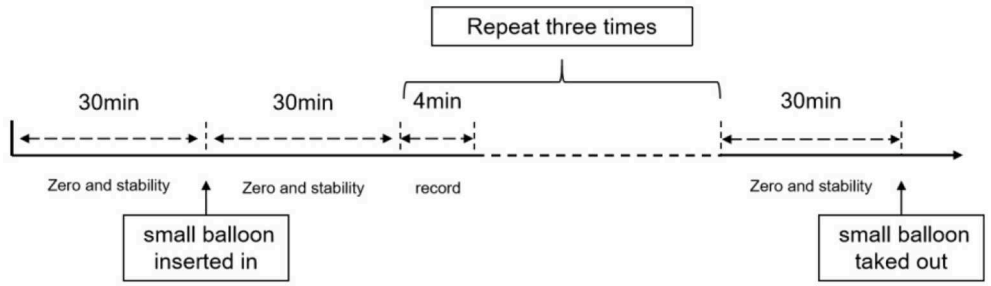


FIGURE 2
Schedule of colonic motion detection.

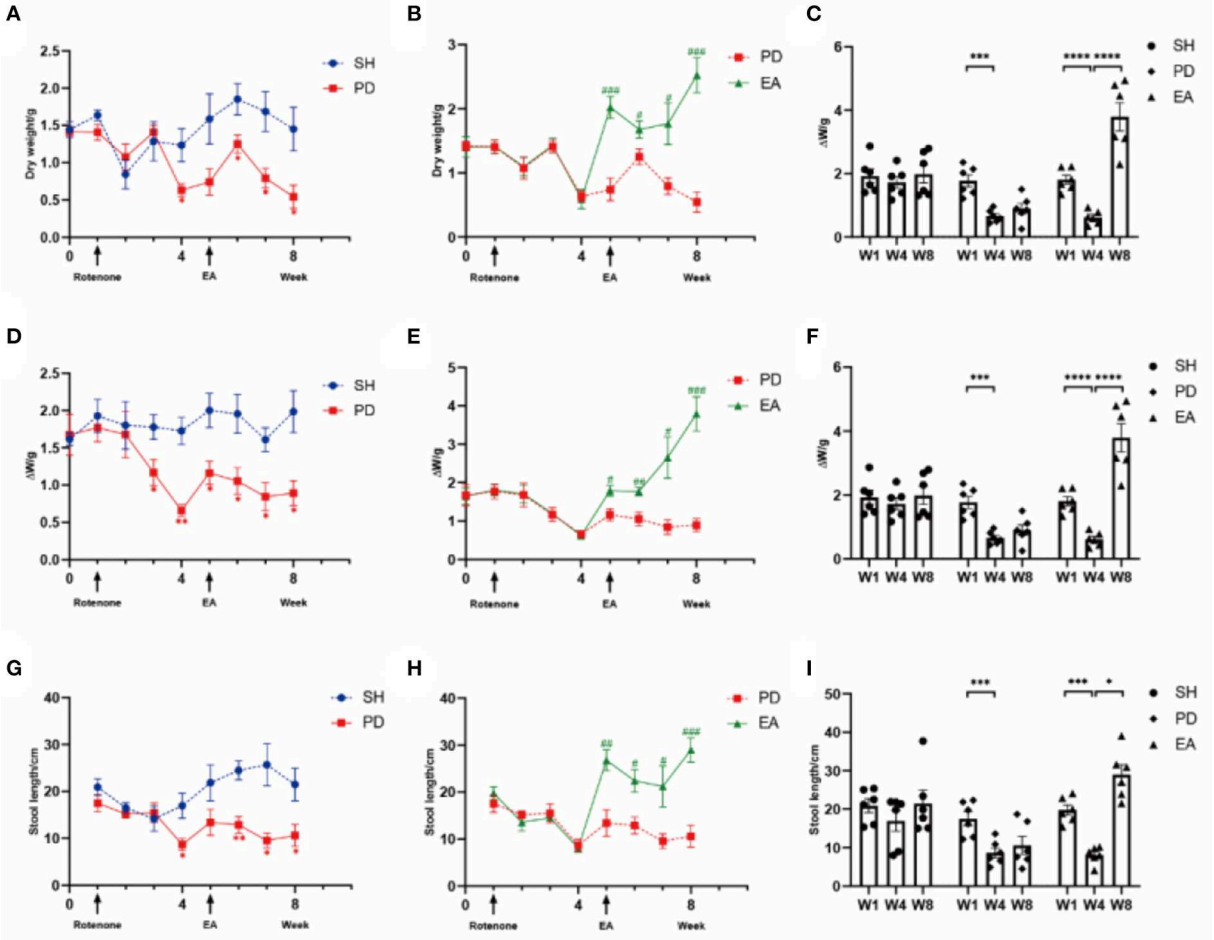


FIGURE 3
Excretion index of rotenone rats in different groups. Levels of **(A)** dry weight (Time × Rotenone Factor, $F = 3.301$, $P < 0.01$), **(D)** ΔW ($F = 2.405$, $P < 0.05$), and **(G)** length ($F = 2.905$, $P = 0.01$) in the SH group and PD group over 8 weeks ($n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$). Compared with the PD group, EA treatment increased **(B)** dry weight (Time × Rotenone Factor, $F = 9.749$, $P < 0.0001$), **(E)** ΔW ($F = 10.02$, $P < 0.0001$), and **(H)** length ($F = 6.068$, $P < 0.0001$) significantly ($n = 6$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$). Comparison of dry weight **(C)**, ΔW **(F)**, and length **(I)** before and after rotenone or EA treatment in each group ($n = 6$, $^{*}p < 0.05$, $^{***}p < 0.001$, and $^{****}p < 0.0001$). W, week; SH, the control group; PD, the model group; EA, the treatment group.

used for comparing two different groups. All data analyses were performed using SPSS 23.0 software (IBM Corp., Armonk, NY, USA), and GraphPad Prism 9.4 (GraphPad Inc., La Holla, CA, USA) was used for data analysis. $p < 0.05$ was considered to indicate statistical significance.

Results

Effect of EA on representative symptoms of PD constipation replicated and whole gut transit time in rotenone rat models

We measured stool dry weight, ΔW, and length to evaluate the successful induction of models. Compared with the SH group, rotenone injection significantly decreased stool dry weight (Figures 3A, C), ΔW (Figures 3D, F), and length (Figures 3G, I). Significant extensions of WGTT (Figure 4) were observed in the PD group, and the stool characteristics became dry, with smaller particles and brown-yellow color, which were consistent with clinical constipation. EA treatment increased stool dry weight (Figures 3B, C), ΔW (Figures 3E, F), length (Figures 3H, I), shortened WGTT (Figure 4), and recovered stool characteristics significantly compared with the PD group. Taken together, stool characteristics, output, and transit time were corrected in the EA group, suggesting the therapeutic effect of EA in PD constipation.

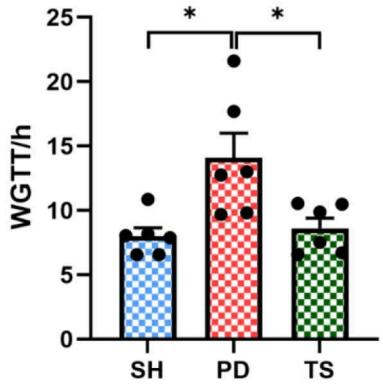


FIGURE 4
Whole gut transit time (WGTT) of rats after solvent, rotenone, and EA treatment ($n = 6$, $^*p < 0.05$).

Effect of EA on colonic motility of rotenone-induced rats

After EA treatment, the balloon method and the multi-conductive physiological recording system were used to record the colonic movement, and the mean pressure and movement frequency [the meaning of "frequency" is the overall frequency of colon activity, which is mainly influenced by meaningless irregular fibrillation (shown by the white arrow), rather than the density of peristaltic waves that represent effective movement.] of the colonic movement were calculated. The peristaltic wave in the SH group was continuous with uniform amplitude (Figure 5A). The PD group had irregular towering malformed waves (shown by the black arrow), and the interval between the two waves increased significantly (Figure 5B), suggesting that the rats in the PD group had intestinal peristalsis rhythm disruption and useless contraction. In the EA group, the continuity of the interval was restored and the abnormal waves were reduced but not completely disappeared (Figure 5C). Compared with the PD group, the colon motility frequency decreased and peristaltic waves per minute increased significantly in the EA group (Figures 5D, E). At the same time, we found that rotenone treatment increased the mean colonic pressure and amplitude of the PD group, while EA treatment did not reverse this change but further increased it (Figures 5F, G). The cause of this phenomenon will be analyzed in the discussion section in combination with the influence of the interference wave shown by the white arrow and the malformation wave shown by the black arrow on colon motion.

After the manual rejection of broad distorted waves, the frequency, pressure, and amplitude are collected and analyzed once more. The results showed that the mean colonic pressure and normal peristaltic wave amplitude decreased in the PD group and recovered after the EA intervention (Figures 6B, C), while the frequency results remain unchanged compared with the pre-adjustment period (Figure 6A). After adjusting the cutoff frequency to 0.1 Hz to exclude the effect of chattering waves, the frequency of the PD group decreased compared with the SH group (Figure 6D), while the mean pressure and amplitude results did not change (Figures 6E, F).

Effect of EA on unmyelinated nerve fiber density and histopathology of colonic myenteric nervous plexus in rotenone rats

We evaluated the effect of EA on the colonic myenteric nervous plexus by observing cross sections of the plexus under electron microscopy. Compared with the control group (Figure 7A), we observed a disturbed nerve structure in the PD group, with edema and sparse numbers of unmyelinated nerve fibers, as well as sparse and disorganized nerve microfilaments and microtubules (Figure 7B). After EA treatment, the nerve structure was improved. Compared with the PD group, the morphology of unmyelinated nerve fibers was more complete, edema was reduced, and the number of nerve filaments and

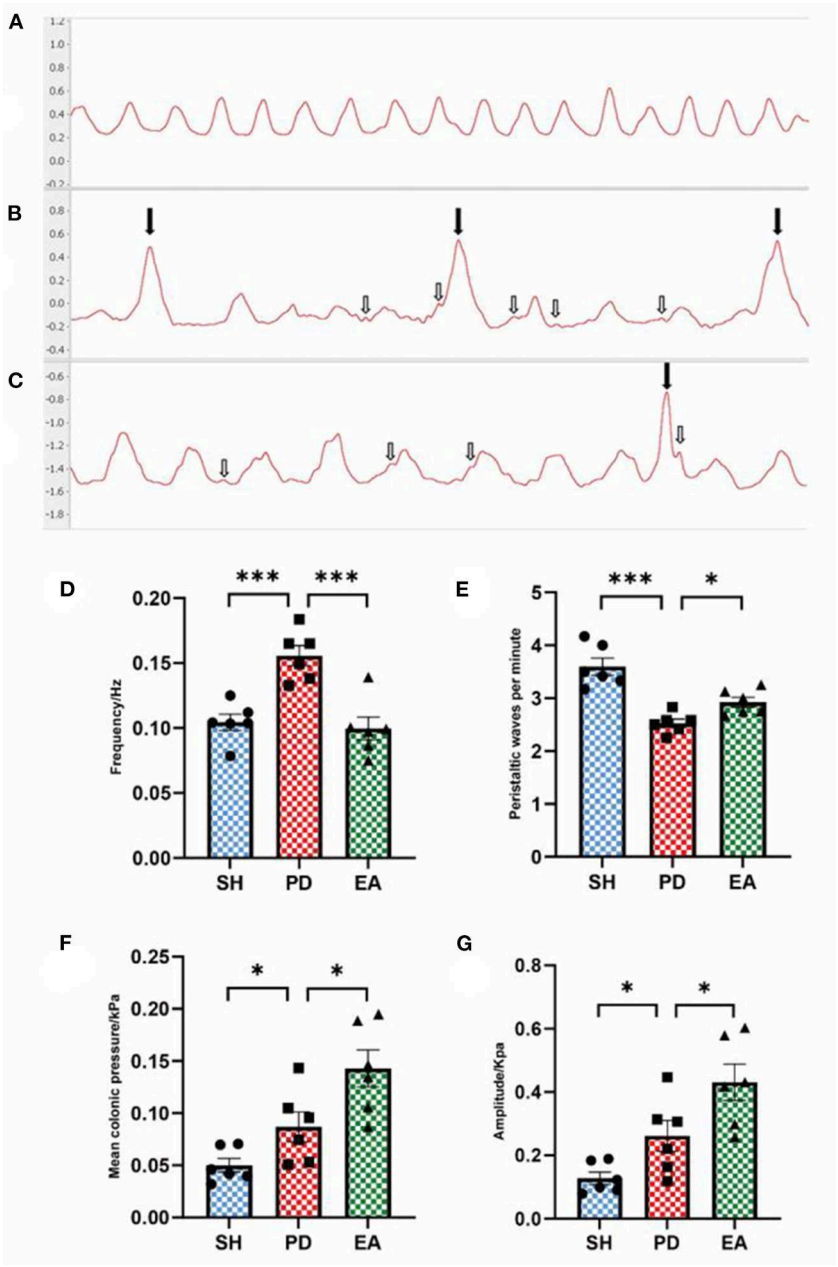


FIGURE 5
Effect of EA on colonic motility. Representative record of colon motion waveform in each group **(A–C)**. The frequency **(D)**, mean pressure **(F)**, and amplitude **(G)** of the PD group increased significantly compared with the SH group, and the peristaltic waves per minute **(E)** decreased. Compared with the PD group, EA treatment decreased the frequency **(C)** and increased the peristaltic waves per minute **(E)**. In contrast, the mean pressure **(F)** and amplitude **(G)** continued to rise ($n = 6$, $^{*}P < 0.05$ and $^{***}p < 0.001$).

microtubules was increased, while the nerve microfilaments and microtubules were neatly arranged (Figure 7C). We further counted the density of unmyelinated nerve fibers and found that the nerve fiber density in the PD group was lower than that in the SH group, while the nerve density increased significantly after EA treatment (Figure 7D).

Effects on excitatory and inhibitory neurons of colonic myenteric nervous plexus

The results of IF in the colonic myenteric nervous plexus showed that nNOS expression in the colonic

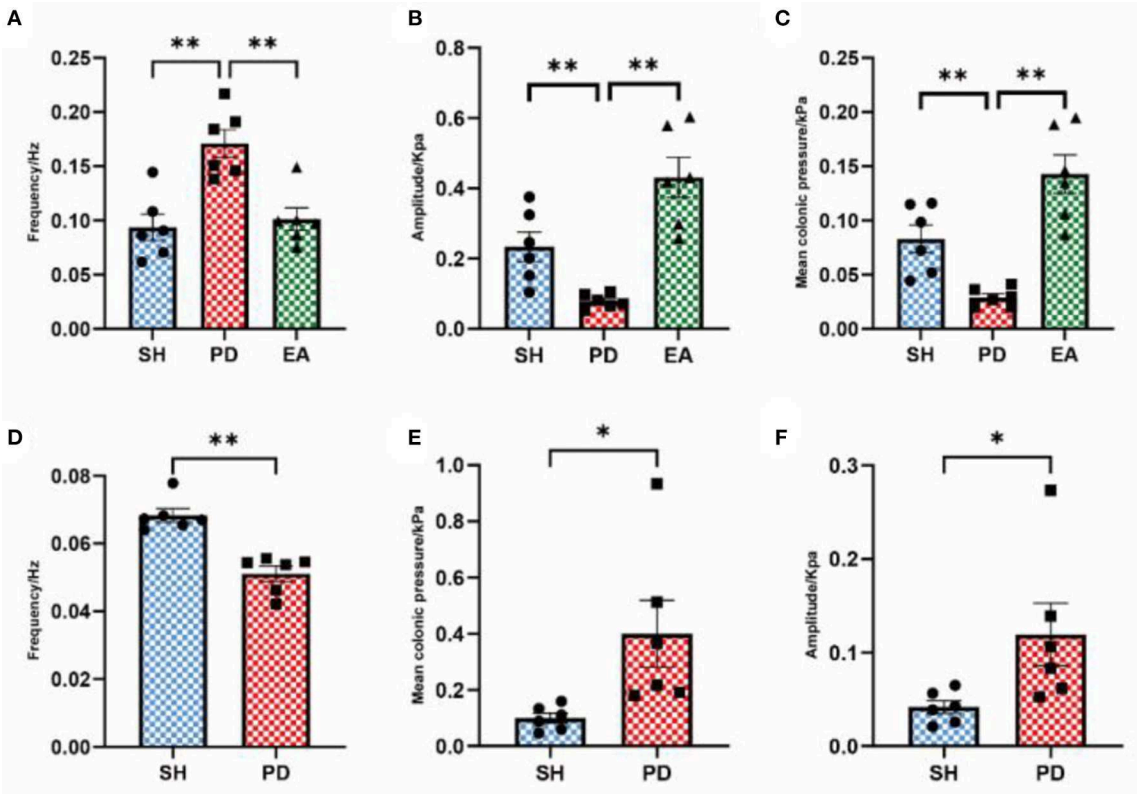


FIGURE 6
Effect of EA on colonic motility after parameter adjustment. The frequency **(A)**, amplitude **(B)**, and mean pressure **(C)** after eliminating the influence of abnormal waves. The frequency **(D)**, amplitude **(E)**, and mean pressure **(F)** after eliminating the influence of the vibration wave ($n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$). The actual meaning of the waveform without vibration wave is the peristaltic wave, which has been analyzed in the form of the peristaltic wave number per minute above (Figure 4E). Therefore, the comparison between the model and EA groups is not carried out here.

myenteric nervous plexus was significantly higher in the PD group compared with the SH group, while EA treatment significantly reduced nNOS expression in the myenteric nervous plexus (Figure 8).

The results of IF in the colonic myenteric nervous plexus showed that ChAT expression in the colonic myenteric nervous plexus was significantly reduced in the PD group compared with the SH group, whereas EA treatment significantly increased ChAT expression in the myenteric nervous plexus (Figure 9).

Effects on the α-syn aggregation of colonic myenteric nervous plexus

The results of IF in the colonic myenteric nervous plexus showed that α-syn aggregation in the colonic myenteric nervous plexus was significantly higher in the PD group compared with the SH group, while EA treatment significantly decreased it (Figure 10).

Discussion

In the present study, we demonstrated that rotenone disrupts ENS structure and function, resulting in prolonged WGTT and decreased mean colonic pressure and normal peristaltic wave density and amplitude in PD rats. Acupuncture treatment ameliorates ENS abnormalities and colonic dysfunction in PD rats, possibly by protecting the structural integrity of the colonic myenteric nervous plexus and balancing neurotransmitter expression.

In addition to typical motor dysfunction, NMS of PD is presented in more than 90% of people with PD (39). One of the key features of the prodromal phase, particularly constipation, is gastrointestinal dysfunction. Constipation is one of the most common and earliest forms of NMS, with a prevalence of up to 80% in people with PD. A total of 5% of patients develop constipation 10–20 years before the appearance of exercise symptoms. In addition, constipation may be a risk factor for PD, as men or women with constipation are two to five times more likely to be diagnosed with PD

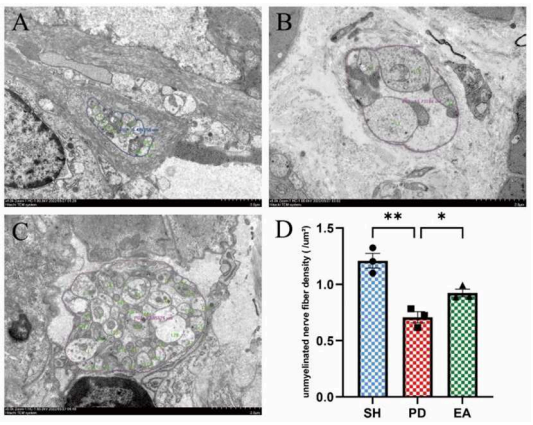


FIGURE 7
Effect of EA on colonic myenteric nervous plexus. Representative myenteric nervous plexus cross section under the transmission electron microscopy (Scale bar, 2 μm) of the control **(A)**, model **(B)**, and EA **(C)** group. Compared with the PD group, EA treatment increased the unmyelinated nerve fiber density **(D)** ($n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$).

in the future (40). Therefore, constipation may be potentially useful for early diagnosis and therapeutic intervention in PD (41). The histology of PD is characterized by the formation of Lewy vesicles and Lewy neurosynapses, consisting mainly of intracellular aggregates of misfolded alpha-synuclein. Louis' vesicles are found in different areas of the brain and areas outside the CNS, such as the ENS (4, 5), and alpha-synuclein lesions can spread between the interconnected areas of the PNS and CNS, that is, the brain–gut axis. In Braak's classic hypothesis of the stages of PD pathogenesis, Lewy body lesions in the submucosal and myenteric nervous plexus neurons are at stage 0 of the disease process, that is, ENS lesions precede cerebral neurodegeneration. This suggests that ENS lesions play a very important role in the pathogenesis of PD. The ENS is the intrinsic nervous system of the gastrointestinal tract and consists of neuronal cell bodies or ganglia arranged in two nerve plexuses. The submucosal plexus is located between the mucosa and the cricothyroid muscle and mainly regulates secretion. The myenteric nervous plexus is located between the cricoid and longitudinal musculature and mainly controls smooth muscle movement. Each nerve plexus contains a complex, heterogeneous population of neurons. Neurons express and often co-express a variety of transmitters and neuropeptides, including acetylcholine, nitric oxide, vasoactive intestinal peptide (VIP), and dopamine (7, 42). The regulation of gastrointestinal function by ENS is due to the release of specific neurotransmitters synthesized by functional neurons. The main inhibitory neurotransmitters involved in the regulation of gastrointestinal motility are nitric oxide and vasoactive intestinal peptide (VIP), while acetylcholine (ACh) is the most represented excitatory neurotransmitter in the entire gastrointestinal tract. The control of bowel function by the ENS is based on the interaction between different neuronal subtypes in the ENS and the balance between the type of input and the level of postsynaptic receptor expression released into the neuronal network by each neurotransmitter (43). ENS degeneration is an important cause of gastrointestinal dysfunction during the prodromal period of PD. Therefore, PD and constipation can be treated with strategies to improve gastrointestinal function.

The efficacy of acupuncture in treating motor (12–14) and NMS, such as cognitive impairment (44), constipation (18, 19), insomnia (17), pain (45), and anxiety (15) in patients with PD has been widely supported by studies. Alternative therapies are reported to be used by 40% of people with PD, with acupuncture being the third most commonly used alternative therapy for PD (46). Acupuncture has a positive effect on relieving constipation symptoms in PD, although evidence from high-quality clinical studies is not yet available (18, 19). In addition to PD constipation, acupuncture is also considered an effective or safe treatment for functional constipation and other gastrointestinal disorders (25, 26). In terms of mechanistic studies, the absence of dopaminergic neurons in the nigrostriatal pathway is the most common pathological factor in PD. Acupuncture can mitigate brain dopaminergic neuronal damage through various pathways, such as apoptotic pathway, autophagic pathway, oxidative stress-related pathway, survival pathway, and neurotransmitter and its receptor and neurotrophic factor expression, thereby achieving neuroprotective mechanisms (15, 28, 29). In recent years, the gut–brain axis has offered a potential entry point for the treatment of PD as the gut–brain axis theory proposes a close connection between the gastrointestinal tract and the central nervous system (47). A growing number of studies have explored the feasibility of acupuncture in the treatment of PD by modulating gastrointestinal function (48). Ma et al. found that EA could improve PD-mediated neuropathy by promoting intestinal barrier repair and reducing intestinal α-syn deposits to inhibit neuroinflammation (49). Jang et al. showed that the enhanced motor function and protective effect of acupuncture on dopaminergic neurons in PD mice may be related to the regulation of gut microbial dysbiosis and thus the suppression of neuroinflammation (50). Another study reported that acupuncture may be beneficial in irritable bowel syndrome by modulating motor, visceral sensory, and/or gut–brain interactions (51). Considering the early damage to the ENS and the central substantia nigra striatal degeneration associated with dopaminergic innervation caused, there are no reports of acupuncture related to ENS lesions. In this context, we aimed to assess the effects of ENS structure and function on colonic motor patterns and associated neurotransmitter control in PD model rats and to explore potential mechanisms of action of acupuncture.

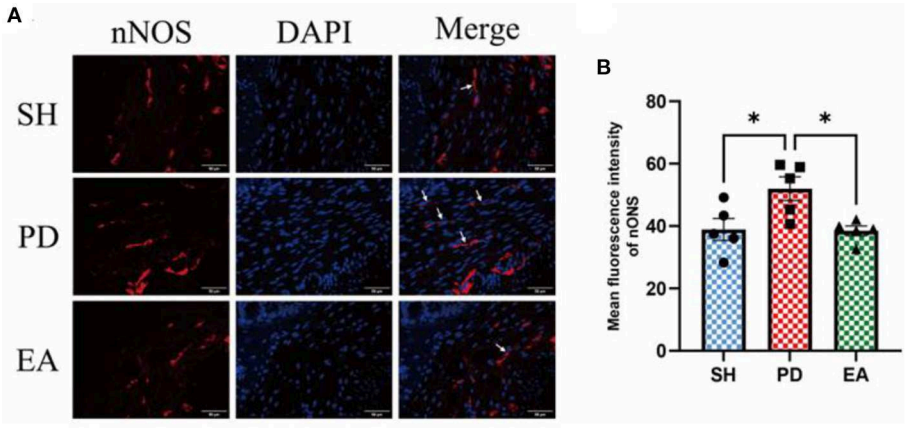


FIGURE 8
(A) Representative IF images of the colon. DAPI stained the nuclei (blue), while the red immunofluorescence represents the nNOS. The colon was observed under a microscope (×400 magnification). Scale bar = 50 μm. The three groups share scale bars. **(B)** Mean fluorescence intensity of nNOS. ($n = 5$, $^{*}p < 0.05$).

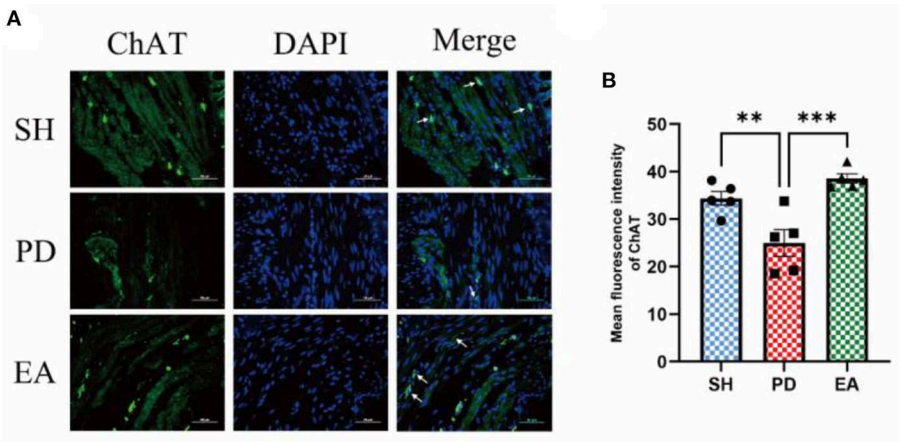


FIGURE 9
(A) Representative IF images of the colon. DAPI stained the nuclei (blue), while the green immunofluorescence represents the ChAT. The colon was observed under a microscope (×400 magnification). Scale bar = 50 μm. The three groups share scale bars. **(B)** Mean fluorescence intensity of ChAT. ($n = 5$, $^{**}p < 0.01$, $^{***}p < 0.001$).

In this study, a model of rotenone induction was recruited to characterize colonic dysfunction and ENS injury. Compared with the MPTP (52) model, rotenone can reproduce the central and gastrointestinal features of PD in rats (53, 54), especially at low doses (2.5 mg/kg) of rotenone treatment, which has been further shown in mice to provide SNpc and myenteric nervous plexus replication of neurodegeneration and the presence of α-syn aggregates (55). Rotenone reliably induces PD models and has an effect on the gastrointestinal tract, which plays a role in the development of PD constipation models. In the present study, abnormal stool characteristics, excretion and water volume (Figure 3), and prolonged whole bowel transit time (WGTT) were observed in rotenone-induced rats (Figure 4), which is consistent with the clinical features of constipation and suggests the successful establishment of the model.

A previous study found *in vitro* that rotenone-treated rats exhibited physiological deficits in inhibitory neurons in the ENS, as evidenced by increased isometric contractility and reduced relaxation of the colonic longitudinal muscles (53). We have also discovered evidence in *in vivo* experiments that is consistent with this. We observed an increase in colonic frequency, mean pressure, and amplitude from the PD group (Figures 5D, F, G), and frequent towering distortion waves (Figure 5B) can be seen (shown by black arrows). Their amplitude exceeds that of normal creeping waves by a factor of more than one, making them easily observable. Another distinctive feature is the marked

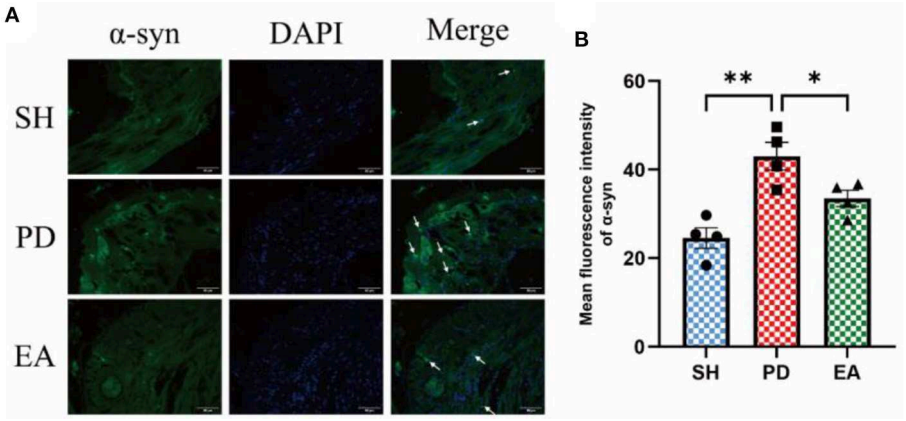


FIGURE 10
(A) Representative IF images of the colon. DAPI stained the nuclei (blue), while the green immunofluorescence represents the α-syn. The colon was observed under a microscope (×400 magnification). Scale bar = 50 μm. The three groups share scale bars. **(B)** Mean fluorescence intensity of α-syn. ($n = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$).

increase in the interval between two adjacent creeping waves (for convenience, we have named it the compensatory interval after the ECG waveform) and the decrease in the amplitude of normal creeping waves (see below for a detailed analysis), which means that the frequency (Figure 5E) and quality (Figure 6C) of valid creeping waves appear less frequently in the same period of time. The actual significance of this elevated distortion wave is not well-understood, as it appears to be a short period of intense bowel contraction. After we had removed this aberrant wave, the data were again collected and analyzed. The results showed that the mean colonic pressure and normal peristaltic wave amplitude in the PD group were reduced and recovered after the EA intervention (Figures 6B, C), while the frequency results were unchanged (Figure 6A). This is another confirmation that the efficiency of bowel movements is reduced in the PD group and is associated with the appearance of aberrant waves.

At the same time, we observed frequent irregular small fibrillation waves (shown by white arrows) in the main wave (creeping wave) and the compensatory interval. The amplitude of these small waves is not sufficiently different from the main wave to increase bowel movements. However, they are much larger than the main waves and are responsible for a large part of the increase in frequency in the PD group. As we observed a decrease in the number of creeping waves per minute in the PD group (Figure 5E), we further adjusted the parameters by adjusting the cutoff frequency to 0.1 Hz to exclude its effect. This suggests that this fluttering wave has a significant effect on the overall frequency of movement of the colon. It increases the energy expenditure of the colonic tissue but does not contribute to the propulsion of the intestinal contents. We hypothesize that it also had an effect on the decrease in mean colonic pressure and normal peristaltic wave amplitude in the PD group (Figures 6B, C). In summary, combining the results of constipation symptoms and whole bowel transit time, we hypothesize that although colonic movements in the PD group were hyperactive, they were mostly ineffective in nature. This is a reflection of a disturbance in the rhythm of bowel movement. The effect of EA on mean colonic pressure and amplitude shows an abnormal increase instead of a decrease (Figures 5F, G), due to the abnormal occurrence of aberrant and fluttering waves, which cause an abnormally high value in the PD group. This represents a pathological state of hyperactive and ineffective contraction in the PD group (Figures 6B, C). Unlike the PD group, the increase in mean colonic pressure in the EA group was not due to ineffective contraction. The increase in the density and amplitude of the peristaltic waveform was the reason for the increase in mean pressure compared with the PD group. As the bowel rhythm is restored, the increase in the index of colonic motility has a positive effect on the improvement of constipation.

To further test this hypothesis, we set our sights on excitatory and inhibitory neurotransmitters in the colonic myenteric nervous plexus. ENS inhibitory neurotransmission is achieved using non-adrenergic non-cholinergic pathways. NO is the main inhibitory neurotransmitter in the ENS and nNOS is the rate control enzyme for its production, mediating smooth muscle relaxation in the gastrointestinal tract, which is important for intestinal motility. Ach is an important excitatory neurotransmitter in the gastrointestinal tract, and ChAT is the main marker of cholinergic structure in the gastrointestinal tract. The IF results showed an increase in the expression of the inhibitory neurotransmitter marker nNOS in the colonic myenteric nervous plexus (Figure 8) and a decrease in the expression of the excitatory neurotransmitter marker ChAT in the PD group (Figure 9). In contrast, EA reversed this alteration. One study found that rotenone treatment did not alter the

number of myenteric nervous plexus neurons (53). We believe that the integrity of the physiological function of the ENS is not only reflected in the number of neurons. The signaling between neurons is also essential. At the same level of neuronal numbers, if intercellular connections are reduced, the ENS is still in a state of reduced function. Therefore, we observed the density of unmyelinated nerve fibers in the myenteric nervous plexus by electron microscopy. The results indicate that rotenone treatment reduced the density of unmyelinated nerve fibers in the PD group, while EA reversed this state (Figure 4). In addition, we found significant α-syn aggregation in the colonic myenteric nervous plexus in the PD group, and EA treatment reversed this state. Although previous studies and our experiments have found that α-syn aggregation is mainly present in the submucosal plexus, α-syn is closely associated with pathological alterations in the myenteric nervous plexus as a major factor contributing to neuronal degeneration in Parkinson's pathology.

Conclusion

In summary, subcutaneous administration of rotenone reproduces the clinical signs of constipation, delayed colonic transit, and ENS abnormalities in PD rats. EA intervention significantly improved stool characteristics and accelerated colonic transit in PD rats. This accelerating effect may be achieved by protecting the structural integrity of the ENS myenteric nervous plexus and balancing ENS excitatory and inhibitory neurons. These findings suggest mechanisms of ENS in PD in gastrointestinal motility disorders and the therapeutic role of acupuncture in PD combined with constipation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Scientific Investigation Board of the Nanjing University of Traditional Chinese Medicine, Nanjing, China (permission number. 202112A047).

Author contributions

X-mH and BX: full access to all of the data in the study and responsibility for the integrity of the data and the accuracy of the data analysis. X-mH, BX, and NX: study concept and design. L-z-xS, XQ, and J-wD: acquisition, analysis, or interpretation of data. L-z-xS and YL: drafting of the manuscript. YL: critical revision of the manuscript for important intellectual content. L-z-xS and J-wD: statistical analysis. X-mH, BX, and ZY: funding, administrative, technical, or material support, and study supervision. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by grants from the National Natural Science Foundation of China (Number. 81904279) and the Natural Science Foundation of Jiangsu Province (Number. BK20190136). The authors' work reported here was also supported by grants from the Nanjing young talents training plan of traditional Chinese Medicine (Number. NJSZYYQNRC-2020-HXM).

Acknowledgments

The authors thank all the grants for this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Warnecke T, Schafer KH, Claus I, Del TK, Jost WH. Gastrointestinal involvement in parkinson's disease: pathophysiology, diagnosis, and management. *NPJ Parkinsons Dis*. (2022) 8:31. doi: 10.1038/s41531-022-00295-x

2. Stirpe P, Hoffman M, Badiali D, Colosimo C. Constipation: an emerging risk factor for parkinson's disease? *Eur J Neurol*. (2016) 23:1606–13. doi: 10.1111/ene.13082

3. Travagli RA, Browning KN, Camilleri M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance. *Nat Rev Gastroenterol Hepatol*. (2020) 17:673–85. doi: 10.1038/s41575-020-0339-z

4. Braak H, Del TK, Rub U, de Vos RA, Jansen SE, Braak E. Staging of brain pathology related to sporadic parkinson's disease. *Neurobiol Aging*. (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9

5. Braak H, de Vos RA, Bohl J, Del TK. Gastric alpha-synuclein immunoreactive inclusions in meissner's and auerbach's plexuses in cases staged for parkinson's disease-related brain pathology. *Neurosci Lett*. (2006) 396:67–72. doi: 10.1016/j.neulet.2005.11.012

6. O'Day C, Finkelstein DI, Diwakarla S, McQuade RM. A critical analysis of intestinal enteric neuron loss and constipation in parkinson's disease. *J Parkinsons Dis*. (2022) 12:1841–61. doi: 10.3233/JPD-223262

7. Furness JB. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol*. (2012) 9:286–94. doi: 10.1038/nrgastro.2012.32

8. Schaffernicht G, Shang Q, Stievenard A, Botzel K, Dening Y, Kempe R, et al. Pathophysiological changes in the enteric nervous system of rotenone-exposed mice as early radiological markers for parkinson's disease. *Front Neurol*. (2021) 12:642604. doi: 10.3389/fneur.2021.642604

9. Prasad EM, Hung SY. Current therapies in clinical trials of parkinson's disease: a 2021 update. *Pharmaceuticals*. (2021) 14:717. doi: 10.3390/ph14080717

10. Angelopoulou E, Anagnostouli M, Chrousos GP, Bougea A. Massage therapy as a complementary treatment for parkinson's disease: a systematic literature review. *Complement Ther Med*. (2020) 49:102340. doi: 10.1016/j.ctim.2020.102340

11. Deuel LM, Seeberger LC. Complementary therapies in parkinson disease: a review of acupuncture, tai chi, qi gong, yoga, and cannabis. *Neurotherapeutics*. (2020) 17:1434–55. doi: 10.1007/s13311-020-00900-y

12. Jang JH, Park S, An J, Choi JD, Seol IC, Park G, et al. Gait disturbance improvement and cerebral cortex rearrangement by acupuncture in parkinson's disease: a pilot assessor-blinded, randomized, controlled, parallel-group trial. *Neurorehabil Neural Repair*. (2020) 34:1111–23. doi: 10.1177/1545968320969942

13. Sun Y, Li L, Chen Y, Wang L, Zhai L, Sheng J, et al. Feasibility and positive effects of scalp acupuncture for modulating motor and cerebral activity in parkinson's disease: a pilot study. *NeuroRehabilitation*. (2022) 51:467–79. doi: 10.3233/NRE-220048

14. Yang LS, Li YM, Zhou DF, Zhao BM, Zheng SZ, Chen ZH, et al. Effects of qihuang needling on motor function for patients with parkinson's disease: study protocol for a multicenter, randomized controlled trial. *Front Neurol*. (2022) 13:902170. doi: 10.3389/fneur.2022.902170

15. Fan JQ, Lu WJ, Tan WQ, Liu X, Wang YT, Wang NB, et al. Effectiveness of acupuncture for anxiety among patients with parkinson disease: a randomized clinical trial. *JAMA Netw Open*. (2022) 5:e2232133. doi: 10.1001/jamanetworkopen.2022.32133

16. Huang CH, Lin MC, Hsieh CL. Acupuncture treatment reduces incidence of parkinson's disease in patients with depression: a population-based retrospective cohort study in taiwan. *Front Aging Neurosci*. (2020) 12:591640. doi: 10.3389/fnagi.2020.591640

17. Li L, Jin X, Cong W, Du T, Zhang W. Acupuncture in the treatment of parkinson's disease with sleep disorders and dose response. *Biomed Res Int*. (2022) 2022:7403627. doi: 10.1155/2022/7403627

18. Li K, Wang Z, Chen Y, Shen L, Li Z, Wu Y, et al. Efficacy of electroacupuncture for the treatment of constipation in parkinson's disease: study protocol for a multicentre randomised controlled trial. *BMJ Open*. (2019) 9:e29841. doi: 10.1136/bmjopen-2019-029841

19. Lu Z, Lu J. Therapeutic observation of acupuncture-moxibustion plus tuina for tinnitus and hearing loss. *Shanghai J Acupunct Moxibust*. (2017) 36:71–3. doi: 10.13460/j.issn.1005-0957.2017.01.0071

20. Cao L, Li X, Li M, Yao L, Hou L, Zhang W, et al. The effectiveness of acupuncture for parkinson's disease: an overview of systematic reviews. *Complement Ther Med*. (2020) 50:102383. doi: 10.1016/j.ctim.2020.102383

21. Gai C, Qiang T, Zhang Y, Chai Y, Feng W, Sun H. Electroacupuncture in treatment of parkinson disease: a protocol for meta-analysis and systematic review. *Medicine*. (2021) 100:e23010. doi: 10.1097/MD.0000000000023010

22. Huang J, Qin X, Cai X, Huang Y. Effectiveness of acupuncture in the treatment of parkinson's disease: an overview of systematic reviews. *Front Neurol*. (2020) 11:917. doi: 10.3389/fneur.2020.00917

23. Li Q, Wu C, Wang X, Li Z, Hao X, Zhao L, et al. Effect of acupuncture for non-motor symptoms in patients with parkinson's disease: a systematic review and meta-analysis. *Front Aging Neurosci*. (2022) 14:995850. doi: 10.3389/fnagi.2022.995850

24. Xu W, OuYang S, Chi Z, Wang Z, Zhu D, Chen R, et al. Effectiveness and safety of electroacupuncture in treating parkinson disease: a protocol for systematic review and meta-analyses. *Medicine*. (2021) 100:e25095. doi: 10.37766/inplasy2021.2.0031

25. Li H. Acupuncture and regulation of gastrointestinal function. *World J Gastroenterol*. (2015) 21:8304. doi: 10.3748/wjg.v21.i27.8304

26. Liu Z, Yan S, Wu J, He L, Li N, Dong G, et al. Acupuncture for chronic severe functional constipation: a randomized trial. *Ann Intern Med*. (2016) 165:761–9. doi: 10.7326/M.15-3118

27. Fan JQ, Lu WJ, Tan WQ, Feng WC, Zhuang LX. Acupuncture for parkinson's disease: from theory to practice. *Biomed Pharmacother*. (2022) 149:112907. doi: 10.1016/j.biopha.2022.112907

28. Li X, Wei W, Wang Y, Wang Q, Liu Z. Global trend in the research and development of acupuncture treatment on parkinson's disease from 2000 to 2021: a bibliometric analysis. *Front Neurol*. (2022) 13:906317. doi: 10.3389/fneur.2022.906317

29. Tamtaji OR, Naderi TM, Notghi F, Alipoor R, Bouzari R, Asemi Z. The effects of acupuncture and electroacupuncture on parkinson's disease: current status and future perspectives for molecular mechanisms. *J Cell Biochem*. (2019) 120:12156–66. doi: 10.1002/jcb.28654

30. Ko JH, Lee H, Kim SN, Park H. Does acupuncture protect dopamine neurons in parkinson's disease rodent model?: a systematic review and meta-analysis *Front Aging Neurosci*. (2019) 11:102. doi: 10.3389/fnagi.2019.00102

31. Oh JY, Lee YS, Hwang TY, Cho SJ, Jang JH, Ryu Y, et al. Acupuncture regulates symptoms of parkinson's disease via brain neural activity and functional connectivity in mice. *Front Aging Neurosci*. (2022) 14:885396. doi: 10.3389/fnagi.2022.885396

32. Zuo T, Xie M, Yan M, Zhang Z, Tian T, Zhu Y, et al. *In situ* analysis of acupuncture protecting dopaminergic neurons from lipid peroxidative damage in mice of parkinson's disease. *Cell Prolif*. (2022) 55:e13213. doi: 10.1111/cpr.13213

33. Chen X, Zhang N, Zhao H, Mu Y. Relationship between behavioral and nigrostriatal pathological damage in rats with parkinson's disease caused by rotenone. *Chin J Nerv Mental Dis*. (2008) 232–234.

34. Chang Y, Luo X, Ren Y. Nigrostriatal to behavioral and nigrostriatal dopaminergic neuronal damage in rats damaged by rotenone. *Progr Anat Sci*. (2011) 17:60–2. doi: 10.16695/j.cnki.1006-2947.2011.01.019

35. Cuperus FJC, Iemhoff AA, van der Wulp M, Havinga R, Verkade HJ. Acceleration of the gastrointestinal transit by polyethylene glycol effectively treats unconjugated hyperbilirubinaemia in gunn rats. *Gut*. (2010) 59:373–80. doi: 10.1136/gut.2009.183921

36. van der Wulp MYM, Cuperus FJC, Stellaard F, van Dijk TH, Dekker J, Rings EHHM, et al. Laxative treatment with polyethylene glycol does not affect lipid absorption in rats. *J Pediatr Gastroenterol Nutr*. (2012) 55:457–62. doi: 10.1097/MPG.0b013e3182555ba9

37. Wang X, Yang B, Yin J, Wei W, Chen JDZ. Electroacupuncture via chronically implanted electrodes improves gastrointestinal motility by balancing sympathovagal activities in a rat model of constipation. *Am J Physiol Gastrointest Liver Physiol*. (2019) 316:G797–805. doi: 10.1152/ajpgi.00018.2018

38. Wang X, Shi H, Shang H, Su Y, Xin J, He W, et al. Are primo vessels (pvs) on the surface of gastrointestine involved in regulation of gastric motility induced by stimulating acupoints st36 or cv12? *Evid Based Complement Alternat Med*. (2012) 2012:787683. doi: 10.1155/2012/787683

39. Blesa J, Foffani G, Dehay B, Bezard E, Obeso JA. Motor and non-motor circuit disturbances in early parkinson disease: which happens first? *Nat Rev Neurosci*. (2022) 23:115–28. doi: 10.1038/s41583-021-00542-9

40. Gao X, Chen H, Schwarzschild MA, Ascherio A. A prospective study of bowel movement frequency and risk of parkinson's disease. *Am J Epidemiol*. (2011) 174:546–51. doi: 10.1093/aje/kwr119

41. Schapira A, Chaudhuri KR, Jenner P. Non-motor features of parkinson disease. *Nat Rev Neurosci*. (2017) 18:435–50. doi: 10.1038/nrn.2017.62

42. Furness JB. Types of neurons in the enteric nervous system. *J Auton Nerv Syst*. (2000) 81:87–96. doi: 10.1016/S0165-1838(00)00127-2

43. Xu J, Wang L, Chen X, Le W. New understanding on the pathophysiology and treatment of constipation in parkinson's disease. *Front Aging Neurosci*. (2022) 14:917499. doi: 10.3389/fnagi.2022.917499

44. Li Z, Chen J, Cheng J, Huang S, Hu Y, Wu Y, et al. Acupuncture modulates the cerebello-thalamo-cortical circuit and cognitive brain regions in patients of parkinson's disease with tremor. *Front Aging Neurosci*. (2018) 10:206. doi: 10.3389/fnagi.2018.00206

45. Yu SW, Lin SH, Tsai CC, Chaudhuri KR, Huang YC, Chen YS, et al. Acupuncture effect and mechanism for treating pain in patients with parkinson's disease. *Front Neurol*. (2019) 10:1114. doi: 10.3389/fneur.2019.01114

46. Rajendran PR, Thompson RE, Reich SG. The use of alternative therapies by patients with parkinson's disease. *Neurology*. (2001) 57:790–4. doi: 10.1212/WNL.57.5.790

47. van Laar T. Introduction: the gut-brain axis in parkinson's disease. *J Parkinsons Dis*. (2019) 9:S279. doi: 10.3233/JPD-199004

48. Nazarova L, Liu H, Xie H, Wang L, Ding H, An H, et al. Targeting gut-brain axis through scalp-abdominal electroacupuncture in parkinson's disease. *Brain Res*. (2022) 1790:147956. doi: 10.1016/j.brainres.2022.147956

49. Ma X, Wang Q, Yuan W, Wang Y, Zhou F, Kang K, et al. Electroacupuncture alleviates neuroinflammation and motor dysfunction by regulating intestinal barrier function in a mouse model of parkinson disease. *J Neuropathol Exp Neurol*. (2021) 80:844–55. doi: 10.1093/jnen/nlab046

50. Jang JH, Yeom MJ, Ahn S, Oh JY, Ji S, Kim TH, et al. Acupuncture inhibits neuroinflammation and gut microbial dysbiosis in a mouse model of parkinson's disease. *Brain Behav Immun*. (2020) 89:641–55. doi: 10.1016/j.bbi.2020.08.015

51. Chey WD, Maneerattaporn M, Saad R. Pharmacologic and complementary and alternative medicine therapies for irritable bowel syndrome. *Gut Liver*. (2011) 5:253–66. doi: 10.5009/gnl.2011.5.3.253

52. Han N, Kim Y, Ahn S, Hwang T, Lee H, Park H. A comprehensive phenotype of non-motor impairments and distribution of alpha-synuclein deposition in parkinsonism-induced mice by a combination injection of mptp and probenecid. *Front Aging Neurosci*. (2020) 12:599045. doi: 10.3389/fnagi.2020.599045

53. Greene JG, Noorian AR, Srinivasan S. Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of parkinson's disease. *Exp Neurol*. (2009) 218:154–61. doi: 10.1016/j.expneurol.2009.04.023

54. Johnson ME, Stringer A, Bobrovskaya L. Rotenone induces gastrointestinal pathology and microbiota alterations in a rat model of parkinson's disease. *Neurotoxicology*. (2018) 65:174–85. doi: 10.1016/j.neuro.2018.02.013

55. Miyazaki I, Isooka N, Wada K, Kikuoka R, Kitamura Y, Asanuma M. Effects of enteric environmental modification by coffee components on neurodegeneration in rotenone-treated mice. *Cells*. (2019) 8:221. doi: 10.3390/cells8030221

frontiers | Frontiers in Neurology

TYPE Systematic Review
PUBLISHED 26 April 2023
DOI 10.3389/fneur.2023.1100327

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Zhengtang Liu,
China Academy of Chinese Medical Sciences, China
Xuan Zhang,
Hong Kong Baptist University, Hong Kong SAR, China

*CORRESPONDENCE
Yan-ming Xie
✉ ktzu2018@163.com

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 16 November 2022
ACCEPTED 17 March 2023
PUBLISHED 26 April 2023

CITATION
Sun L-x, Li Y-y and Xie Y-m (2023) Efficacy and safety of Tongmai Jiangtang capsule combined with conventional therapy in the treatment of diabetic peripheral neuropathy: a systematic review and meta-analysis.
Front. Neurol. 14:1100327.
doi: 10.3389/fneur.2023.1100327

Efficacy and safety of Tongmai Jiangtang capsule combined with conventional therapy in the treatment of diabetic peripheral neuropathy: a systematic review and meta-analysis

Lin-xi Sun[1,2], Yuan-yuan Li[1] and Yan-ming Xie[1]*

[1]Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing, China, [2]Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Background: Recently, more and more Chinese patent drugs have been proved to be effective in the treatment of diabetic peripheral neuropathy (DPN). Tongmai Jiangtang capsule (TJC) is one of the representative ones. The present meta-analysis integrated data from several independent studies to determine the efficacy and safety of TJCs combined with routine hypoglycemic therapy for DPN patients, and to evaluate the quality of evidence.

Methods: SinoMed, Cochrane Library, PubMed, EMBASE, Web of Science, CNKI, Wanfang, VIP databases and registers were searched for randomized controlled trials (RCTs) involving TJC treatment of DPN up to February 18, 2023. Two researchers independently used the Cochrane risk bias tool and comprehensive reporting criteria for Chinese medicine trials to evaluate the methodological quality and reporting quality of the qualified studies. RevMan5.4 was used for Meta-analysis and evidence evaluation, with scores determined for recommendations, evaluation, development and GRADE. The Cochrane Collaboration ROB tool was used to evaluate the quality of the literature. The results of Meta-analysis were represented by forest plots.

Results: A total of 8 studies were included involving a total sample size of 656 cases. TJCs combined with conventional treatment (CT) could significantly accelerate myoelectricity graphic nerve conduction velocity, including that median nerve motor conduction velocity was faster than those of CT alone [mean difference (MD) = 5.20, 95% confidence interval (CI): 4.31–6.10, $P < 0.00001$], peroneal nerve motor conduction velocity was faster than those of CT alone (MD = 2.66, 95% CI: 1.63–3.68; $P < 0.00001$), median nerve sensory conduction velocity was faster than those of CT alone (MD = 3.06, 95% CI: 2.32–3.81, $P < 0.00001$), and peroneal nerve sensory conduction velocity was faster than those of CT alone (MD = 4.23, 95% CI: 3.30–5.16, $P < 0.00001$). The total efficiency of the TJCs + CT group was higher than that of the CT group (RR = 1.41, 95% CI: 1.28–1.56, $P < 0.00001$). The HbA1c after treatment in the TJCs + CT group was lower than that in the CT group ($P < 0.05$). No adverse drug reactions (ADRs) were reported in the combined TJCs or CT groups.

Conclusions: TJCs combined with CT reduced the severity of DPN symptoms and no treatment-associated ADRs were reported. However, these results should be considered with caution because there was marked heterogeneity in the research data. Therefore, more stringent RCTs should be designed to validate the efficacy of TJCs in DPN patients.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=264522, identifier: CRD42021264522.

KEYWORDS

diabetic peripheral neuropathy, Tongmai Jiangtang capsule, meta-analysis, system evaluation, traditional Chinese medicine

1. Introduction

Diabetes mellitus is a chronic disease affecting the health and quality of life of a significant proportion of the population. One of the common debilitating complications of diabetes involves DPN, a symmetrical form of peripheral neuropathy. As diabetes develops, DPN begins as abnormal sensations but can progress to disabling neuropathic pain. According to national DPN screening conducted by the Diabetes Society of Chinese Medical Association, the prevalence of DPN in China was as high as 52.97% (1), with clinical symptoms mainly reported as abnormal sensations and movement of the extremity. The symptoms usually start from the toes and developed to the proximal extremities, showing a "glove and sock" distribution with muscle weakness often found in the late stage of the disease. About one third of patients with DPN will develop pain in their limbs, variously described by sufferers as burning or stabbing pain, electrical shock-like or cutting pain (2, 3). The lower extremity paresthesia caused by DPN is the primary cause of foot ulceration and even amputation, which greatly reduces the quality of life of patients and causes a huge social and economic burden.

At present, the pathogenesis of DPN has not been fully clarified, but it is generally known that the generation of nerve injury and pain is related to metabolic disorders, loss of nerve fibers, oxidative stress, inflammatory responses, genetic factors and the influence of lifestyle. Hu and Yang (4) proposed that DPN is caused by multiple factors associated with hyperglycemia including metabolic disorders, vascular damage, neurotrophic disorders, oxidative stress and genetic factors, resulting in activation of various metabolic pathways including the polyol pathway, hexosylamine pathway, protein kinase C pathway and also the formation of glycation end products. Xu et al. (5) suggested there were two main causes of the disease, namely, a lack of blood supply and pathophysiological changes in neurons or nerve fibers caused by high blood glucose. Oxidative stress is also key factor in many chronic complications of diabetes, including DPN. The current therapeutic goals of DPN are to relieve symptoms and prevent the development and progression of neuropathy. The various drug therapies used aim to normalize hyperglycemia, hypertension, and hyperlipidemia, to correct antioxidant stress, and to improve microcirculation and induce nerve repair. In this respect, traditional Chinese medicines and patent Chinese medicines have become important auxiliary methods for the clinical management of DPN.

TJCs are a kind of Chinese medicine to nourish Yin and clear heat, mainly comprised of *Radix Pseudostellariae*, *Radix Salviae Miltiorrhizae*, *Rhizoma Coptidis*, *Radix Astragali*, *Gynostemma pentaphylla*, *Rhizoma Atractylodis*, *Radix Scrophulariae*, *Hirudo*, *Fructus Malvae*, *Radix Puerariae*, and *Rhizoma Dioscoreae*. TJCs have the effect of nourishing the Yin and clearing heat and promoting blood circulation and can be used to treat diabetes caused by deficiency of qi and Yin and blood stasis of veins (6). Although clinical studies have reported that TJCs can be used adjuvant treatment in DPN, the evidence is relatively scattered and no relevant systematic review has presently been conducted to properly evaluate the quality of evidence. Therefore, the primary aim of this study was to address this problem by conducting a systematic review of the current literature. Toward this, literature that meets the quality standards was systematically and strictly screened to achieve an accurate evaluation of the effectiveness and safety of TJCs combined with Western medicine in the treatment of DPN. This study therefore provides an up-to-date reference for the clinical application of TJCs in DPN.

Abbreviations: TJC, Tongmai Jiangtang capsule; DPN, diabetic peripheral neuropathy; RCT, randomized controlled trial; CBM, Chinese BioMedical Database; CNKI, Chinese National Knowledge Infrastructure; CT, Conventional treatment of western medicine; MD, mean difference; CI, confidence interval; RR, risk ratio; ADE, adverse event; ADR, adverse reaction; TNF-α, tumor necrosis factor; IL-6, interleukin 6; SC, schwann cells; AGES, advanced glycation end products; BDNF, brain-derived nerve growth factor; NGF, nerve growth factor.

2. Methods

2.1. Study registration

This systematic review was registered in the PROSPERO (registration number: CRD42021264522), and referred to the "PRISMA 2020 Checklist" (http://www.prisma-statement.org) (7).

2.2. Search strategy

We searched the following eight databases and two register websites from their inception to February 18, 2023: PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure, Chinese BioMedical Database, WanFang Database, VIP Database (VIP), https://clinicaltrials.gov/, and http://www.chictr.org.cn/. The following terms were searched in the abstract or title of the study: (Diabetic peripheral neuropathy OR peripheral neuropathy OR DPN) AND (Tongmai Jiangtang capsule OR Tongmai hypoglycemic capsule OR Tongmai Jiangtang) AND (clinical research OR clinical observation curative effect observation OR clinical efficacy OR clinical evaluation OR clinical trial OR RCT, etc), and the comprehensive search of subject words combined with free words is carried out based on the respective characteristics of the database. Take PubMed as an example, the detailed search strategy was shown in Supplementary Table 1. The detailed retrieval search strategies we have developed for each database are in Supplementary material. We also manually searched for studies that met the inclusion criteria from other sources not included in the above database, as well as for dissertations, conference papers, such as gray literature, and unpublished research results from relevant companies. Two researchers (Sun LX and Li YY) independently screened eligible studies, and identified inconsistencies by discussing them with other researchers. The studies were retrieved in Chinese and English languages.

2.3. Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) only the RCTs was involved; (2) patients meeting diagnostic criteria for DPN: a diagnosis of diabetes (2), with arms and legs (at least in the double lower limbs) with persistent pain and/or sensory disturbance, abatement of vibratory sensation in both thumbs or at least one thumb, double side or side of the ankle reflex decreased to disappear, myoelectricity graphic double lower limbs nerve conduction velocity delay, median nerve, phil total nerve, motor nerve conduction velocity (MNCV) < 40 m/s, sensory nerve conduction velocity (SNCV) of sural nerve < 45 m/s, prolonged latency, decreased amplitude and other electrophysiological abnormalities (8, 9); (3) treatment groups were treated using oral TJCs combined with CT, according to the general treatment and etiological treatment methods in the Guidelines for the Prevention and Treatment of Type 2 Diabetes in China (10) (2020 edition) and control groups treated with CT (conventional drugs including mecobalamine, etc); Conventional drugs for the treatment of DPN refer to Table 1. (4) main outcome of median nerve motor nerve conduction velocity; secondary outcomes of peroneal nerve motor conduction velocity, median nerve sensory conduction velocity, and peroneal nerve sensory conduction velocity; and safety outcome of any ADR. The exclusion criteria were as follows: (1) clinical summative literature only; (2) studies in which data were incomplete or cannot be extracted, and republished literature; (3) the data had obvious errors; (4) incomparable objective outcome indicators.

2.4. Data extraction and risk of bias assessment

According to the standard information extraction table, the data was extracted independently by two researchers (Sun LX and Li YY). Throughout the process, differences were resolved through discussion or the participation of another researchers (Xie YM). The basic information extracted from articles included author's name, year of publication, type of study design, number of cases, gender, age, time from symptom onset to randomization, course of treatment, investigator group, treatment regimen, and outcome measures.

Two reviewers (Sun LX and Li YY) independently assessed the risk of bias in each study using the criteria outlined in

TABLE 1 Conventional drugs for the treatment of DPN.

Drug	NNT	AAN	NICE	EFNS	NeuPSIG IASP
GABA analogs				First line	First line
Pregabalin	5.0	First line	First line		
Gabapentin	6.0		First line		
TCAs				First line	
Amitriptyline	1.3	Second line	First line		
Imipramine	2.2	Second line			First line
Desipramine	2.6				First line
SNRIs		Second line		First line	First line
Duloxetine	5.0		First line		
Venlafaxine	3.1				

AAN, American Academy of Neurology; EFNS, European Federation of Neurological Societies; IENFD, intraepidermal nerve fiber density; NeuPSIG IASP, Neuropathic Pain Special Interest Group of the International Association for the Study of Pain; NICE, National Institute for Health and Care Excellence; NNT, Number Needed to Treat for at least 50% pain relief.
Quoted from Khdour Maher (11).

the Cochrane Handbook for Systematic Reviews of Interventions (2019). Disagreements were judged by discussion or participation of another author (Xie YM). The quality of the literature was evaluated using the "bias risk assessment tool" recommended by the Cochrane Collaboration, which included the following domains: (1) randomization; (2) attrition bias; (3) allocation concealments; (4) blinding of participants and personnel; (5) blinding of outcome assessment; (6) integrity of outcome data; (7) selective reporting of study results; (8) other sources of bias. Each potential source of bias was described as "low," "high," and "unclear" (lack of relevant information or uncertainty of bias). The red part of the bias risk table represents high risk, green represents low risk, and yellow represents uncertain risk. Annotations were added to tables when information about the risk of bias and unpublished data sources were supplemented by contacting trial authors. We also included the risk of bias for each study, which may have influenced the outcome, in evaluating treatment effectiveness. Moreover, we used the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) criteria to rank the quality of the evidence using the GRADE profiler (GRADEpro) software (12).

2.5. Data synthesis and analysis

RevMan 5.4 software (provided by the Cochrane Collaboration) was used to perform the meta-analysis and produce the risk of bias graph and risk of bias summary. For dichotomous data, risk ratios (RRs), Mantel Haenszel tests, and 95% CIs analysis were used. Continuous variables were expressed as MD, effect value and 95% CI. Statistical heterogeneity tests were performed with I^2 values ranging from 0% to 100%: $I^2 \leq 40\%$, were considered to have good statistical homogeneity, and the fixed effect model was adopted; $30\% < I^2 < 60\%$, mean moderate heterogeneity; $50\% < I^2 < 100\%$, show substantial statistical heterogeneity, and further analysis was undertaken to assess the reasons of the heterogeneity using subgroup and sensitivity analyses. And the random effects model was used in cases of obvious clinical heterogeneity. Meta-analysis was used to minimize potential clinical heterogeneity, and to identify the reasons for the heterogeneity, including curative effect evaluation standards, index, age, gender and conclusion group intervention measures, such as controlled drugs. For *P*-values of < 0.05, the difference was considered to be statistically

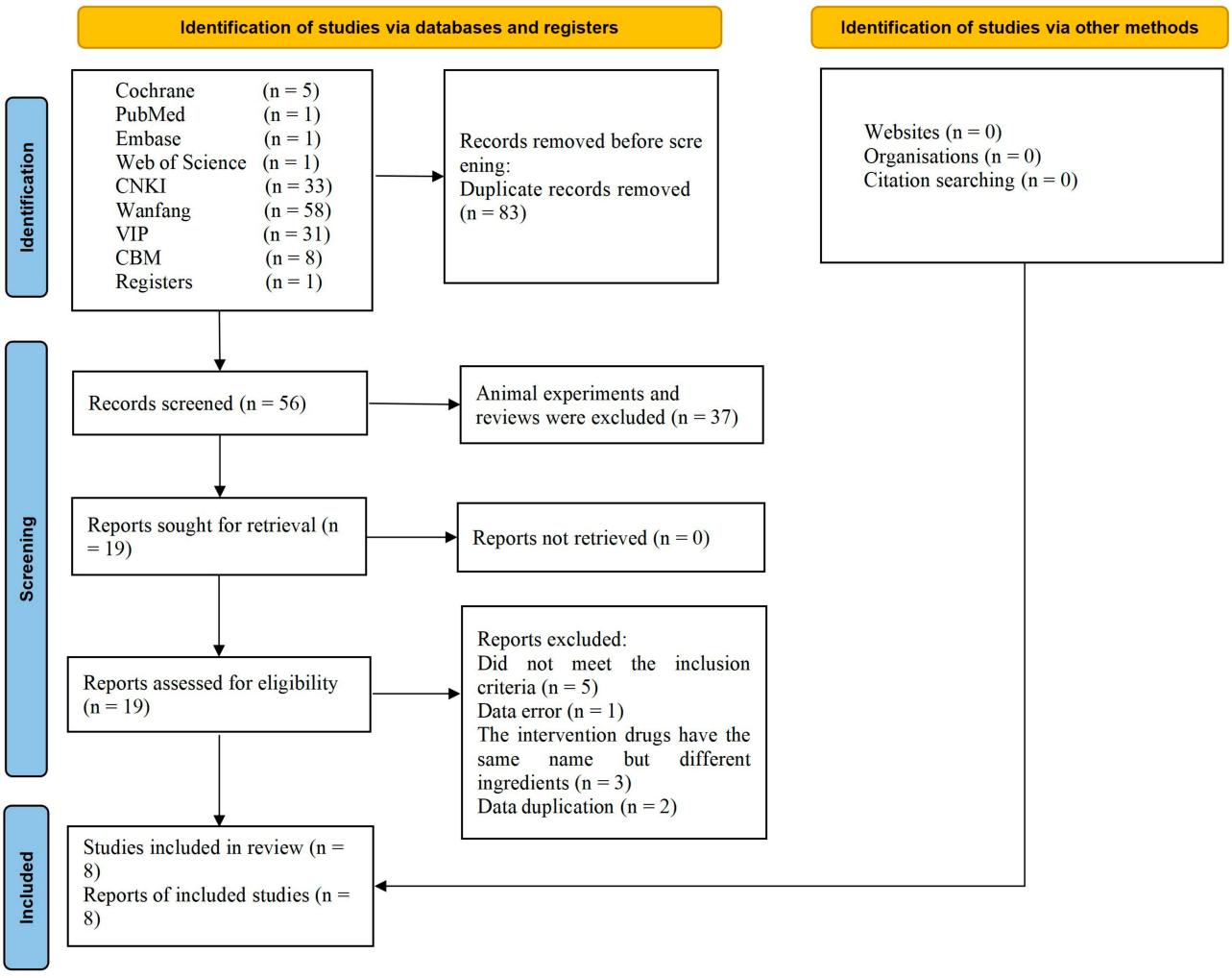


FIGURE 1
PRISMA flow diagram indicating the selection process for this meta-analysis.

significant. Meta-analysis results were presented as forest plots, and possible publication bias was analyzed by funnel plots. If the study was not suitable for meta-analysis, descriptive analysis was performed. If ≥10 articles were included in an outcome index, funnel plots were used to analyze whether publication bias existed.

3. Results

3.1. Search results and study characteristics

A total of 139 studies were identified using the search strategy, including 5 articles from Cochrane library, 1 from PubMed, Embase and Web of Science, 33 from CNKI, 58 from Wanfang, 31 from VIP, 8 from CBM, 1 from register websites. Analysis of these for duplicate studies excluded a total of 83 studies. After reviewing the abstracts, another 37 studies were excluded for not meeting the inclusion criteria. Of the remaining 19 studies, 11 were excluded after full text review. Ultimately, 8 RCTs involving 656 cases were included (13–20). The flowchart of the screening process is presented in Figure 1. Two researchers independently extracted the data from the literature. There were 333 cases in the treatment group and 323 cases in the control group. All 8 RCTs were published in Chinese language between 2010 and 2015. The trial design for all 8 studies involved TJCs combined with CT (mecobalamin, etc.) vs. CT. Treatment courses ranged from 28 to 90 days. Five studies reported ADRs (15–19). The baseline characteristics were consistent across the studies. The detailed characteristics of the studies are presented in Table 2.

3.2. Methodological quality assessment

Risk assessment of bias for the seven trials is shown in Figure 2. All studies were randomly grouped, one used a random number table, and no specific randomization method was reported for the remaining trials. None of the eight studies provided blind information and assignment concealment, and it was not clear whether the randomly assigned investigator and assignment concealment investigator were third-party personnel. As a result, the studies were rated as having ambiguous risks. One study reported in detail the number and reasons for exclusions, and seven studies reported results based on preset outcome indicators; thus all were rated as low risk in terms of outcome data completeness and selective reporting. All studies showed no significant other biases and were therefore rated as low risk.

We should also note that we attempted to contact the published author of each study by phone or email to ask for details of the trial to assess the methodological quality in greater detail but did not receive any responses. It is therefore unclear whether the included studies used strict operating procedures such as allocation, concealment and blinding. Given the overall methodological quality of the included studies was considered poor, the results of this study must be viewed in this context.

4. Primary outcome

4.1. Median nerve motor conduction velocity

Six studies involving 531 cases reported the median nerve median nerve motor conduction velocity (MNCV) (13–18). The forest plot showed significant differences between the TJCs + CT groups and the CT groups (MD = 4.69, 95% CI: 3.48–5.89, $P < 0.00001$; Figure 3), and the heterogeneity was high ($P = 0.08$, $I^2 = 50\%$). The random effect model was selected to analyze the data according to heterogeneity. The results suggested that the MNCV of the median nerve was faster in the TJCs + CT group than in the CT group after treatment.

5. Secondary outcomes

5.1. Peroneal nerve motor conduction velocity (MNCV)

Five studies involving 436 cases reported the peroneal nerve MNCV (13–18). The forest plot (Figure 4) showed significant differences between the TJCs + CT groups and the CT groups (MD = 3.88, 95% CI: 2.45–5.32, $P < 0.00001$). Moreover, it has high heterogeneity ($P = 0.01$, $I^2 = 69\%$). The random effects model was used to analyze the data according to heterogeneity. The results suggested that the MNCV of the peroneal nerve was faster in the TJCs + CT group than in the CT group after treatment.

5.2. Median nerve sensory conduction velocity (SNCV)

Five studies involving 436 cases reported the median nerve SNCV (13–18). The forest plot (Figure 5) showed significant differences between the TJCs + CT groups and the CT groups (MD = 3.66, 95% CI: 2.52–4.80, $P < 0.00001$). Moreover, it has high heterogeneity ($P = 0.03$, $I^2 = 62\%$). The random effects model was used to analyze the data according to heterogeneity. The results suggested that the SNCV of the median nerve was faster in the TJCs + CT group than in the CT group after treatment.

5.3. Peroneal sensory conduction velocity (SNCV)

Five studies involving 436 cases reported the peroneal nerve SNCV (13–18). The forest plot (Figure 6) showed significant differences between the TJCs + CT groups and the CT groups (MD = 3.76, 95% CI: 2.56–4.97, $P < 0.00001$). Moreover, it has high heterogeneity ($P = 0.06$, $I^2 = 56\%$). The random effects model was used to analyze the data according to heterogeneity. The results suggested that the SNCV of the peroneal nerve was faster in the TJCs + CT group than in the CT group after treatment.

TABLE 2 Characteristics of included studies.

Study id (location)	Cases		Gender (male/female)	Age	Course of disease	Intervening measure	Course of treatment	Treatment period	Outcome indicator	Randomization	Double Blinding
	T	C	T/C	T/C	T/C	T	C				
Du (13) (China)	50	40	T: 28/22	T: 51.9 ± 10.1	T: 4.1 ± 1.8 years	TJCs 1.2g, tid + CT	Mecobalamine 1,000 ug, qd, iv drop	56 d	1234	MWD	N.R
			C: 25/15	C: 52.1 ± 9.8	C: 6.0 ± 2.1 years						
Mo (14) (China)	30	30	T: 16/14	T: 48–73 (58.81 ± 7.50)	T: 7–17 years	TJCs 1.2 g, tid + CT	α-lipoic acid 600 mg, qd, iv drop + Mecobalamine 500 ug, qd, iv	28 d	12345	MWD	N.R
			C: 14/16	C: 47–73 (59.12 ± 8.03)	C: 7–16 years						
Kong (15) (China)	60	60	T: 34/26	T: 53–81 (67.9 ± 5.8)	NA	TJCs1.2 g, tid + CT	Mecobalamine 500 ug tid, po	60 d	123457	MWD	N.R
			C: 32/28	C: 53–81 (67.3 ± 4.6)							
Xu and Chen (16) (China)	50	50	T: 24/24 (out of 2 cases)	T: 44–70 (55.0 ± 3.6)	T: 9 months—22 years (5.8 ± 4.6 years)	TJCs 0.8 g, tid + CT	Mecobalamine 500 ug, tid, po	60 d	2357	Random number table	N.R
			C: 22/25 (out of 3 cases)	C: 42–68 (53.3 ± 4.8)	C: 7 months—19 years (6.7 ± 3.4 years)						
Chen et al. (19) (China)	30	30	T: 17/13	T: 37–81	T: 19 cases ≥12 months, 11 cases < 12 months	TJCs 1.2 g, tid + CT	Insulin glargine 8 U, qd, ih + Acarbose 50 mg, tid + Mecobalamine 0.5 mg, tid	90 d	567	MWD	N.R
			C: 16/14	C: 36–80	C: 18 cases ≥12 months, 12 cases < 12 months						
Zhang et al. (17) (China)	53	53	T: 32/21	T: 35–70	T: 12–36 months	TJCs 1.2 g, tid + CT	Mecobalamine 500 ug tid po	90 d	123457	MWD	N.R
			C: 31/22	C: 33–70	C: 10–32 months						
Wang (18) (China)	30	30	T: 16/14	T: 50–70	T: 10 months—6 years	TJCs 1.2 g, tid + original hypoglycemic regimen (details unknown)	Mecobalamine 500 ug, tid, po + original hypoglycemic regimen (details unknown)	90 d	123457	MWD	N.R
			C: 17/13	C: 49–71	C: 9 months—6 years						
Hao et al. (20) (China)	30	30	T:17/13	T: 29–78 (52.12 ± 3.24)	T: 2–10 (4.84 ± 2.22) years	TJCs 1.2 g, tid + CT	Vitamin B1 (100 mg) and B12 (500 μg), im, qd	60 d	5	MWD	N.R
			C:14/16	C: 28–77 (52.36 ± 2.54)	C: 2–11 (4.78 ± 2.15) years						

T, experimental group; C, control group; NA, no available; The outcomes are Median nerve motor conduction velocity, Peroneal nerve motor conduction velocity, Median nerve sensory conduction velocity, Peroneal sensory conduction velocity, total effective rate, glycosylated hemoglobin and adverse reactions, that are denoted by "1" "2" "3" "4" "5" "6" and "7" respectively; MWD, mentioned without description; N.R, not reported.

10.3389/fneur.2023.1100327

FIGURE 2
Assessment of risk of bias in the eight trials. **(A)** Risk bias results proportional chart, **(B)** Summary chart of risk bias results.

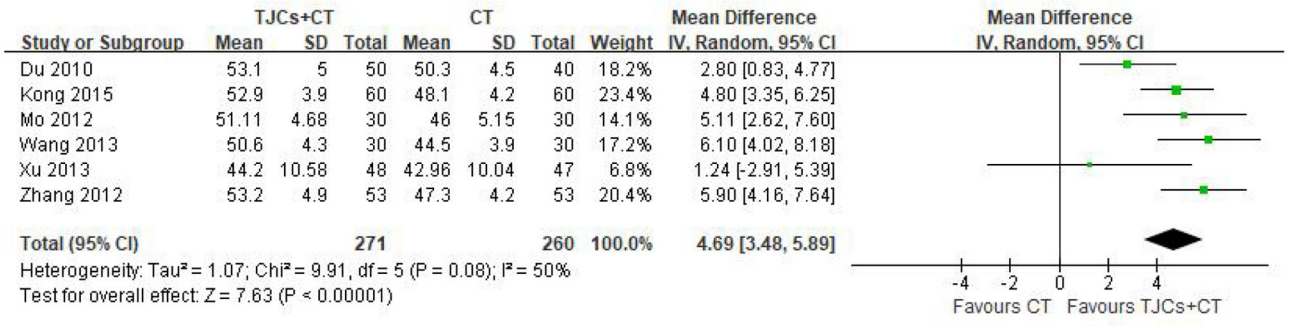


FIGURE 3
Forest plot of Median nerve MNCV (m/s).

5.4. Total effective rate

Seven studies involving 561 cases reported the total effective rate (14–20). The forest plot (Figure 7) showed significant differences between the TJCs + CT groups and the CT groups (RR = 1.41, 95% CI: 1.28–1.56, $P < 0.00001$). Moreover, it has low heterogeneity ($P = 0.29$, $I^2 = 18\%$). The fixed effects model was used to analyze the data according to heterogeneity. The results suggested that the total efficiency of the TJCs + CT group was higher than that of the CT group.

5.5. Glycosylated hemoglobin (HbA1c)

One study involving 60 cases reported HbA1c (19). The HbA1c after treatment was lower than that before treatment in both groups, and the HbA1c after treatment in the TJCs +

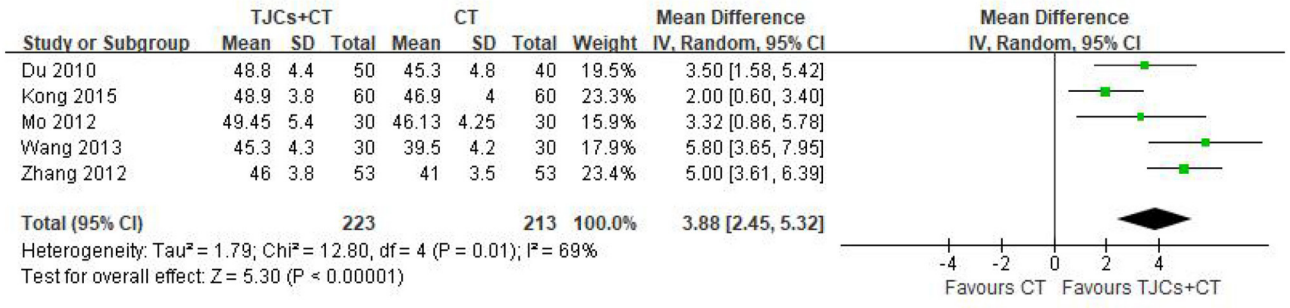


FIGURE 4
Forest plot of Peroneal nerve MNCV (m/s).

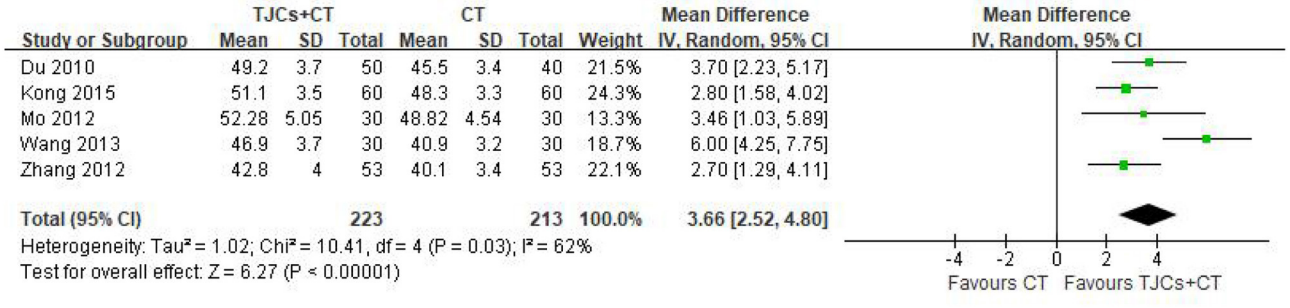


FIGURE 5
Forest plot of Median nerve SNCV (m/s).

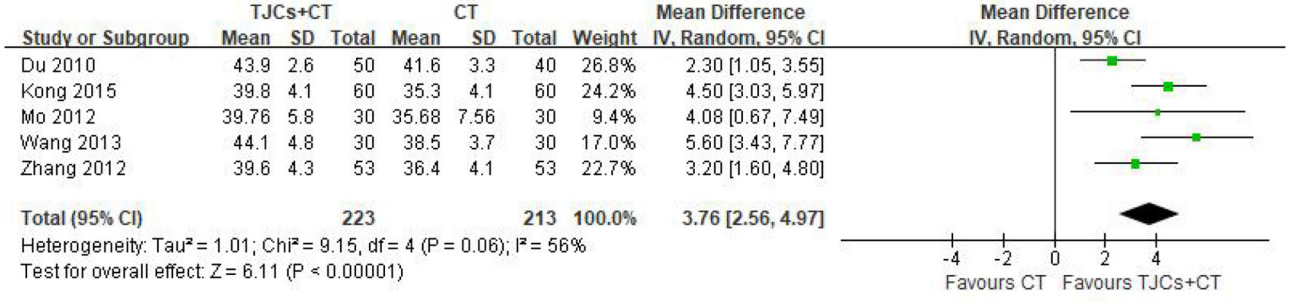


FIGURE 6
Forest plot of Peroneal nerve SNCV (m/s).

CT group was lower than that in the CT group ($P < 0.05$) (Table 3).

5.6. Safety analysis: ADRs

5 literatures involving 446 cases (15–19) were included to report the safety of medication, and all the experimental groups were TJCs combined with CT. There were no ADRs in the experimental group and control group in the 5 clinical observations.

5.7. Sensitivity analysis

After deleting individual studies one by one we observed the changes of I^2 (Table 4), conducted sensitivity analysis, and then explored the potential sources of heterogeneity. With regard to median nerve MNCV, the heterogeneity was the lowest ($P = 0.27$, $I^2 = 22\%$) after removal of the study of Du, which may be related to the inconsistent sample sizes between the experimental and control groups in this study. For peroneal nerve MNCV, the heterogeneity was the lowest ($P = 0.39$, $I^2 = 0\%$) after removal of the study of Wang Qing and Zhang Aiqi, which may be

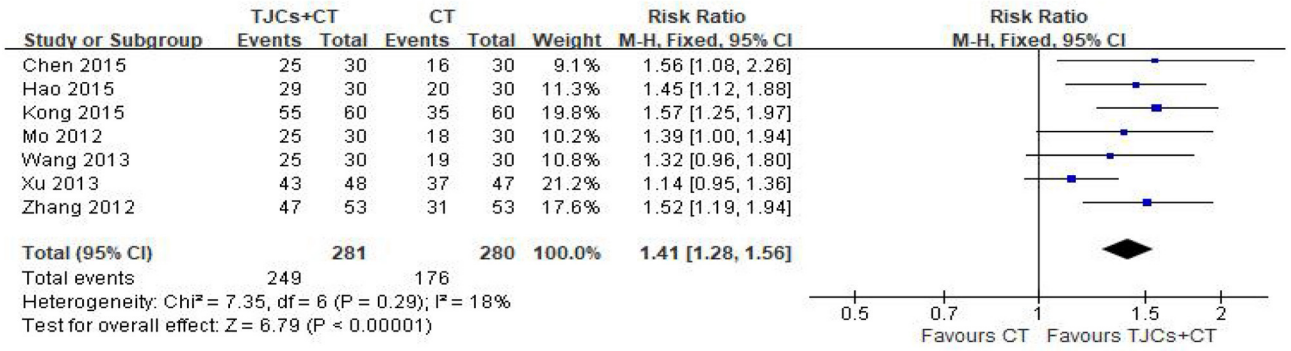


FIGURE 7
Forest plot of total effective rate.

related to the fact that the original hypoglycemic regimen of the patients in the experimental group and control group combined in Wang Qing's study was not detailed. Similarly, the heterogeneity of median nerve SNCV was lowest ($P = 0.74$, $I^2 = 0\%$) after removal of the Wang study, again likely related to specificity issues concerning the hypoglycemic regimen in the experimental and control groups. For peroneal nerve SNCV, the heterogeneity was the lowest ($P = 0.35$, $I^2 = 8\%$) after removal of the study of Du, which again may be related to the inconsistent sample sizes between the experimental and control groups. The heterogeneity results did change to some extent.

5.8. Evaluation of publication bias

Less than 10 studies were included for each outcome index, which did not meet the requirements for making a funnel plot. Hence there was no need to conduct publication bias analysis for the main outcome index.

5.9. Grading quality of evidence

The GRADEpro (www.gradepro.org) has been used to conduct evaluation on the convincing level of evidence and strength of recommendations for the involved outcomes. Initially, RCTs were considered to have high confidence, and cohort studies low confidence as for the estimate of effect. Factors which may have decreased the level of confidence level included inconsistency, limitations, imprecision, indirectness, as well as publication bias. As only RCTs could be included in the review, the reasons for the update (large effects, dose-response relationships and plausible confounders) do not apply. We present the results of GRADE analysis in Table 5.

6. Sample size evaluation

Trial sequential analysis (TSA) for systematic review or meta-analysis sample size estimation overcomes the shortcomings of classical systematic review or meta-analysis (21). First, when the number of cases included in a meta-analysis does not reach a sufficient sample size, the application of TSA minimizes the false positive results due to random errors results. Second, meta-analysis is a retrospective study, and the required information size (RIS) obtained by TSA refers to the number of cases needed to obtain statistically significant differences in meta-analysis, which is the sample size of meta-analysis. The sample size required for meta-analysis is generally considered to be no less than that required for a well-designed, statistically robust, single randomized controlled trial. Again, meta-analysis aims to find evidence of the efficacy of medical interventions as early as possible, and TSA provides a termination criterion for clinical trials by estimating RIS, thus avoiding waste of research and medical resources.

TABLE 3 HbA1c of TJCs + CT vs. CT (%).

Chen et al. (20)	TJCs + CT ($n = 30$)	CT ($n = 30$)
Pre-treatment	8.16 ± 1.04	7.93 ± 1.17
Post-treatment	$6.28 \pm 0.12^{*\#}$	$7.02 \pm 0.57^{*\#}$

Compared with the same index before treatment in the same group, $^{*}P < 0.05$; compared with the same index after treatment in the control group, $^{\#}P < 0.05$.

TSA was used to estimate the sample size of the seven studies reporting the total effective rate. The results of TSA analysis (Figure 8) showed that the cumulative Z value after the first study crossed the traditional cut-off curve ($Z = 1.96$) and crossed the TSA cut-off after the 6th study, indicating that although the cumulative information size did not reach the desired value, no more trials were needed to obtain a positive conclusion in advance (22).

7. Discussion

As far as we know, this study is the first systematic review of the effect of TJCs combined with conventional therapy in the treatment of DPN, and the quality of the evidence was assessed using GRADEpro. The TSA was used to calculate the sample size to estimate the effect more conservatively. The primary objective findings were that, compared with CT alone, the MNCV and

TABLE 4 Heterogeneity and MD in sensitivity analysis of outcome indicators.

Exclusion study	Median nerve MNCV		Peroneal nerve MNCV		Median nerve SNCV		Peroneal nerve SNCV	
	Pooled MD (95% CI)	Heterogeneity value; I^2	Pooled MD (95% CI)	Heterogeneity value; I^2	Pooled MD (95% CI)	Heterogeneity value; I^2	Pooled MD (95% CI)	Heterogeneity value; I^2
Du (13) (China)	5.20 (4.31, 6.10)	0.27; 22%	3.99 (2.17, 5.81)	0.005; 76%	3.68 (2.17, 5.19)	0.02; 71%	4.23 (3.30, 5.16)	0.35; 8%
Mo (14) (China)	4.58 (3.15, 6.01)	0.04; 59%	4.00 (2.28, 5.72)	0.005; 76%	3.71 (2.36, 5.06)	0.02; 71%	3.75 (2.38, 5.12)	0.03; 67%
Kong (15) (China)	4.58 (2.97, 6.20)	0.04; 60%	4.56 (3.64, 5.49)	0.28; 21%	3.94 (2.51, 5.38)	0.04; 65%	3.55 (2.10, 5.00)	0.07; 57%
Xu and Chen (16) (China)	4.94 (4.11, 5.76)	0.14; 43%	/	/	/	/	/	/
Chen et al. (19) (China)	/	/	/	/	/	/	/	/
Zhang et al. (17) (China)	4.49 (3.57, 5.40)	0.09; 49%	3.54 (1.91, 5.18)	0.04; 65%	3.94 (2.54, 5.35)	0.03; 66%	3.98 (2.37, 5.59)	0.03; 66%
Wang (18) (China)	4.38 (3.04, 5.73)	0.09; 51%	3.47 (1.98, 4.95)	0.03; 66%	3.06 (2.32, 3.81)	0.74; 0%	3.27 (2.47, 4.06)	0.16; 43%

TABLE 5 GRADE quality grading evaluation.

Certainty assessment							No. of patients		Absolute (95% CI)	Certainty
Outcomes	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	T	C		
Median nerve MNCV	RCTs	Very serious[a]	Not serious	Not serious	Not serious	Strongly suspected[bc]	271	260	MD 4.69 higher (3.48 higher to 5.89 higher)	⨁◯◯◯ Very low
Peroneal nerve MNCV	RCTs	Very serious[a]	Not serious	Not serious	Not serious	Strongly suspected[bc]	223	213	MD 3.88 higher (2.45 higher to 5.32 higher)	⨁◯◯◯ Very low
Median nerve SNCV	RCTs	Very serious[a]	Not serious	Not serious	Serious	Strongly suspected[bc]	223	213	MD 3.66 higher (2.52 higher to 4.8 higher)	⨁◯◯◯ Very low
Peroneal nerve SNCV	RCTs	Very serious[a]	Not serious	Not serious	Serious	Strongly suspected[bc]	223	213	MD 3.54 higher (2.8 higher to 4.29 higher)	⨁◯◯◯ Very low

CI, confidence interval; RR, relative risks.

[a]Poor methodology, including the method of randomization and blinding; [b]small sample sizes; [c]All results were positive.

10.3389/fneur.2023.1100327

SNCV of median and peroneal nerves were faster when Tongmai Jiangtang capsules were combined with CT, suggesting that TJCs play an active role in the treatment of DPN. However, the heterogeneity among these studies was high. In addition, the quality of research methodology was low, perhaps because of the disjointed comparisons in research designs between different selections of Western medicines and dosage forms. In addition, most of the studies did not mention specific randomization methods, consequently reducing the baseline comparability between groups, which may lead to selective bias (23). Furthermore, the studies did not mention the implementation of blinding methods, which did not exclude the implementation and measurement bias resulting in increased inter-group heterogeneity. Considering the possible bias and large heterogeneity, the effectiveness of TJCs in the treatment of DPN needs to be confirmed by more evidence-based research.

Chinese medicine theory of diabetes argues that the disease does not heal rapidly, which consumes body fluid, and this injury consumes qi. Qi deficiency affects the smooth flow of blood, resulting in blood stasis. Qi and blood cannot run to the end of the limbs, tendons and veins. In short, the pathogenesis of the disease is deficiency of Qi, blood, Yin and Yang, which causes blood stasis, and blood stasis is basis for inducing and accelerating the development of DPN. Thus, DPN marks a basic deficiency involving qi, Yin, and associated blood stasis (24). Indeed, cluster analysis by Pan et al. (25) showed that the pathogenesis of the main syndrome of DPN was mainly characterized by blood stasis, which was related to the spleen and kidney, and mainly manifested as deficiency of qi and Yang in the spleen and kidney. Notably, the patients diet can damage the spleen and stomach, lung and kidney, and the resulting qi deficiency can obstruct blood and promote blood stasis. Protracted spleen and kidney qi deficiency will eventually involve yuan Yang, cold appearance, and thus cause Yang deficiency of the spleen and kidney. Therefore, invigorating qi and nourishing Yin to promote blood circulation and remove blood stasis is one of the important measures for treating DPN.

The components of TJCs are diverse, with each component promoting different mechanisms of action to help treat diabetes and reverse the underlying causes of DPN. In terms of drug composition, *Radix scrophulariae* and *Radix astragalus* can replenish qi and nourish Yin, acting to both lower and regulate blood sugar. Here they act to promote blood circulation, improve microcirculation, improve the hemorheology index and improve blood supply to microvessels to help nurture nerves (26, 27). With *Rhizoma coptidis*, *Radix pseudostellariae*, *Hirudo*, and *Radix puerariae* as matching herbs, TJCs have the effects of supplementing qi, promoting blood circulation and producing fluid. *Salvia miltiorrhiza* and *Hirudo* can also inhibit platelet aggregation, reduce blood viscosity, dilate blood vessels, improve microcirculation, thereby increasing blood supply, oxygenation and nutrition in peripheral limb nerves, promoting the repair of injured peripheral nerves, and improving motor nerve conduction speed (18, 28). *Rhizoma Dioscoreae* and *Gyundum pentaphyllum* have the effects of promoting blood circulation and removing blood stasis, strengthening kidneys and nourishing essence, strengthening the spleen and nourishing the lung, regulating lipids and lowering blood sugar (14, 19). *Fructus Malvae* has a diuretic effect and assisted by *Rhizoma Atractylodis* can dispel wind and stasis dredge collaterals, disperse cold and promote dehumidification (29). Lastly, *Radix Puerariae* improves insulin resistance in patients with type 2 diabetes through a multiplicity of effects, protecting vascular endothelium and endothelial function, improving the hemorheology index, microcirculation and the central nervous function of diabetic patients.

In summary, the combined use of various drugs found in TJCs can promote Yin, clear heat, invigorate qi, invigorate the spleen, remove dampness, and promote blood circulation. The resulting effects treat both the symptoms and root causes of DPN. Moreover, these effects are in line with TCM's drug selection for differentiation of DPN syndrome. This also reflects the advantages of this treatment approach and underscores the ability of TCM to shows synergistic effects when combined with CT.

Along with clinical use, the activity and mechanism of Chinese herbal medicine components including TJCs has also been evaluated in experimental model systems. Investigations by Zhang et al. (30) showed that TJCs could inhibit the expression of inflammatory factors such as tumor necrosis factor (TNF-α) and interleukin 6 (IL-6). Moreover, they showed that TJCs directly act on Schwann cells (SC) in peripheral nerves, significantly inhibiting SC apoptosis induced by advanced glycation end products (AGES) by down-regulating TNF-α and IL-6 mRNA and protein expression. Interestingly, TJCs produced no significant effects on the expression of brain-derived nerve growth factor (BDNF) and nerve growth factor (NGF), proposing that the effects of TJCs in DPN involve a protective role through delaying SC apoptosis. The authors suggested that the mechanism of action of TJCs on SC may be closely related to inflammation theory of the pathogenesis of diabetes. Notably, this study confirms that TJCs not only act on microcirculation but also elicit direct effects on nerve cells.

A network-based pharmacological mechanism study of *Radix scrophulariae* in the treatment of diabetic foot disease found that the main active component was β-sitosterol, followed by salicinol, radix scrophulariae glycosides, and glutosterol in that order. Previous studies have shown that β-sitosterol is a phytosterol with a structure similar to cholesterol. Notably, in a rat model of diabetes induced by a high-fat and sucrose diet, β-sitosterol was shown to attenuate insulin resistance in adipose tissue by modulating the IRS-1/Akt signaling pathway (31). *Radix scrophulariae* can also induce regulatory T cells in humans through increasing the expression of Foxp3, transforming growth factor-β and IL-10 mRNA in resting peripheral blood mononuclear cells (27), therefore highlighting its potential as an anti-inflammatory agent. Furthermore, *Radix Astragali* was shown to significantly improved blood glucose levels in diabetic mice, and also to significantly reduce renal cell injury and glomerular cell apoptosis. This occurred through reductions in the levels of inflammatory response by a mechanism related to activation of the protease-activated receptor 2 (PAR2) signaling pathway (32). Other work showed the key signaling pathways targeted by the combination of *Rhizoma Atractylodis* and *Radix scrophulariae* included the regulation of lipolysis in adipocytes, PPAR signaling pathway, insulin resistance, and the AMPK signaling pathway. The identity of these signaling pathways strongly suggests that the mechanism of DPN treatment may be also related to the regulation of lipolysis

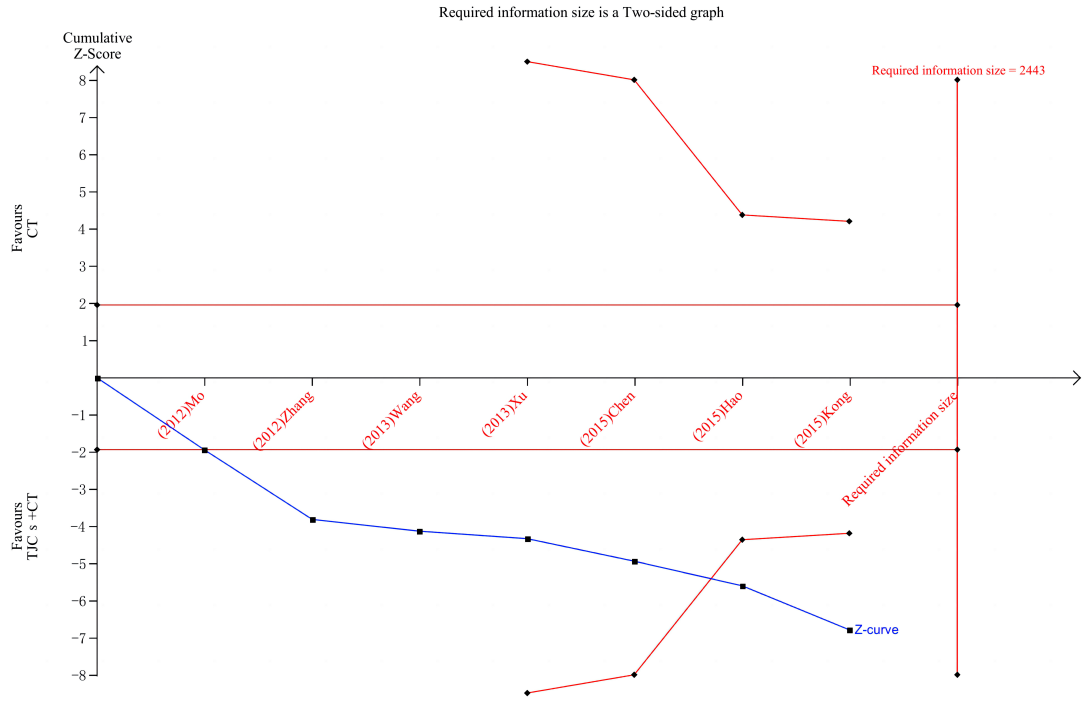


FIGURE 8
TSA of the total effective rate of TJCs + CT in the treatment of DPN. Red curve, TSA boundaries; Red vertical line, required information size; Blue curve, Cumulative Z-values; Black dashed line, Traditional cut-off values.

(33). The combination of *Radix Astragali* with *Radix Puerariae* can also lower blood glucose with optimal effects, with evidence provided to suggest its hypoglycemic mechanism of action may be related to regulating adipokines and mediating AMPK/GLUT-4 transactivation (34).

The pharmacodynamic mechanism of TCM is now being clarified through the combination of ancient theory and modern technology. This approach provides strong guidance for the application of multi-target Chinese patent medicines in the treatment of clinical syndromes such DPN. In this study, we conducted a systematic evaluation based on the reported clinical literature. This provided the conclusion that the efficacy of clinical combination with TJCs in the treatment of DPN was better than that of symptomatic treatment alone. Regardless, such combinations still needs to be applied in accordance with their indications and functional specifications. However, among the included studies, five of eight documented safety results with notably no ADRs reported in either the experimental or control groups. Importantly this indicates that TJCs have no obvious toxicity and side effects, further suggesting that the combination TJCs with CT is safe and suitable for prolonged treatment use. Nevertheless, considering that the ADRs in the drug instructions are not clear, more clinical trials are needed to provide evidence to support the safety evaluation of TJCs in order to achieve clinical standardization and rational safe drug use.

The limitations and prospects of this study are as follows: (1) there are limitations with respect to region and race given the study sites of all evaluated trials were limited to China, and the subject race was not specified; thus the effect of "people and places" was ignored; (2) the research involved different combinations of drug choice, dosage and form. Thus, drug usage was not necessarily the same, particularly for the Western treatments where different medicines and administration methods were used, e.g., insulin and glucosaminic acid-cobalt chelate (35). (3) The included studies did not specifically mention the occurrence of severe syndromes or complications for invalid cases; (4) most studies did not carry out TCM syndrome differentiation for diabetic peripheral neuropathy, and ignored the efficacy of TJC in treating qi and Yin deficiency and vein stasis syndrome; (5) as the quality of the methodology was generally not high, which affects the reliability of the conclusions, clinicians should carefully consider the results of this study accordingly (36). Therefore, it is suggested that in clinical study design, disease differentiation and syndrome differentiation should be combined according to drug instructions to make clinical drug use more accurate and reasonable.

The implications based on the results of this study are as follows: (1) combining TJCs with CT in the treatment of DPN has a good overall effect. It is suggested that TJCs can be added to the clinical treatment of DPN, TJCs can be added on the basis of CT, while paying attention to ADRs; (2) the generally low methodological quality highlights the need for more rigorous research involving larger samples sizes with studies conducted properly in multicenter settings. In particular, there should be strict implementation of randomized controlled trial quality standards to study the effects of TJCs in the treatment of DPN in order to improve the quality levels of evidence for both efficacy and safety (37, 38). This will provide strong evidence and improved guidance for using TJCs in clinical practice; (3) currently, there is

still a lack of uniform clinical norms for the outcome indicators of DPN treatment with Chinese patent medicine, and the total clinical response rate as an outcome indicator has not been internationally recognized (39). To increase the comparability between trials, it is suggested that future studies should first consider MNCV and SNCV as the main outcome indicators for efficacy evaluation; (4) proprietary Chinese medicines follow the ideas of TCM syndrome differentiation and treatment. On this basis, future research should be conducted accordingly with a focus on qi and Yin deficiency, and vein stasis resistance. This will help rule out deficiencies in theoretical guidance that might otherwise lead to poor curative effect of proprietary Chinese medicine in the clinic; (5) in the evaluation of outcome indicators, attention should be paid to exploring the long-term therapeutic effect of TJCs, particularly to increase the examination of objective indicators during follow-up; (6) it is suggested that researchers should pay more attention to the quality of future randomized controlled trials, referring to the standard protocol items of the SPIRIT statement (40). Such research should be carried out in strict accordance with this scheme, with trials featuring a normative design, scheme registration and strengthening of ADR monitoring. The reporting of the results should refer to the CONSORT Statement (Consolidated Standards of Reporting Trials, CONSORT) (41) to further evaluate the clinical efficacy and safety of TJCs.

Finally, we should note that a random effects model was used for meta-combination of four outcome data, mainly because the RCTs of each study did not have unified control group of hypoglycemic drugs, and the quality control of the RCTs was not sufficiently strict. Future RCTs should consider stratifying patients by key factors as much as possible, for example, by age and disease course, etc. In addition, more RCTs should be registered on standardized clinical trial platforms to describe the testing process in detail, so that studies can be more accurate and rigorous.

8. Conclusion

Our Meta-analysis investigated the efficacy and safety of TJCs combined with CT in the treatment of DPN, providing evidence to support the use of combination therapy. However, since most of the included studies were of low quality and the analysis results showed high heterogeneity, there is limited evidence to support this conclusion. This deficiency needs to be addressed in future high-quality, well-designed, multi-center RCTs. Moreover, future clinical studies should focus on improving objectivity in outcome measurements and methodological quality by adopting a rigorous experimental design. This research should strictly follow unified registration procedures and standards to provide higher levels of evidence for clinical applications of TJCs.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Concept design: Y-mX and L-xS. Literature screening, data collection, and integration inspection: L-xS and Y-yL. Manuscript writing: L-xS. Data analysis and interpretation and final review: All authors. All authors contributed to the final draft of the article.

Funding

This work was supported by National Key R&D Program of China, No. 2018YFC1707400.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1100327/full#supplementary-material

References

1. Lu Y, Lu YL. Research progress on the mechanism of diabetic peripheral neuropathy. *J Shanghai Jiaotong Univ.* (2017) 37:1441–5. doi: 10.3969/j.issn.1674-8115.2017.10.027

2. Lin L, Li J, Lin J, Tang S, Li Y. Effectiveness and safety of low-level laser therapy in diabetic peripheral neuropathy: a protocol for a systematic review and meta-analysis. *Syst Rev.* (2021) 10:1–7. doi: 10.1186/s13643-021-01656-y

3. Aniawanis M, Munirah S, Athirah A. The relationship between painful diabetic peripheral neuropathy and functional status of older people in Kuantan, Pahang. *Enferm Clin.* (2021) 31:S130–4. doi: 10.1016/j.enfcli.2020.12.007

4. Hu RM, Yang Z. Impaired pancreatic beta cell function is a key factor in type 2 diabetes: implications from genetic research. *Chin J Diabetes Mellitu.* (2009) 1:86–8. doi: 10.3760/cma.j.issn.16745809.2009.02.003

5. Xu C. The pathogenesis and prevention of diabetes mellitus after organ transplantation. *Armed Police Med.* (2014) 25:757–60. doi: 10.14010/j.cnki.wjyx.2014.08.013

6. Yang W-Q, Yu Y-B, Xu X-L, Ren H-X, Zhang L. Effect of tongmai jiangtang capsule on diabetic peripheral neuropathy in rats. *Chin J Integr Trad Western Med.* (2016) 36:831–4. doi: 10.7661/CJIM.2016.07.0831

7. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372:*n*71. doi: 10.1136/bmj.n71

8. Lyu CY. *Clinical and Mechanism of Tangbikang Intervention in Diabetic Peripheral Neuropathy*. Beijing: University of Chinese Medicine (2014).

9. Gao YB, Zhou H, Zhang TJ, Feng XZ, Guan S, Yi WM, et al. Clinical study of tangluoling in the treatment of diabetic peripheral neuropathy. *Chin J Trad Chin Med.* (2013) 28:1673–7.

10. Diabetes Society of Chinese Medical Association. Guideline for the prevention and treatment of type 2 diabetes mellitus in China (2020 edition). *Chin J Endocrinol Metab*. (2021) 37:311–98. doi: 10.3760/cma.j.cn311282-20210304-00142

11. Khdour Maher R. Treatment of diabetic peripheral neuropathy: a review. *J Pharm Pharmacol*. (2020) 7:13241. doi: 10.1111/jphp.13241

12. Schünemann HJ, Oxman AD, Higgins JP, Vist GE, Glasziou P, Guyatt GH, et al. *Cochrane Handbook for Systematic Reviews of Interventions. The Cochrane Collaboration. Chapter 11: Presenting Results and 'Summary of Findings' Tables*. (2011). Available onlie at: https://handbook-5-1.cochrane.org/chapter_11/11_presenting_results_and_summary_of_findings_tables.htm (accessed January 13, 2023).

13. Du YF. Clinical observation on 50 cases of diabetic peripheral neuropathy treated with mecobalamin combined with Tongmai Jiangtang Capsule. *Chin J Trad Chin Med.* (2010) 51:186. doi: 10.13288/j.11-2166/r.2010.s2.236

14. Mo GG. Tong Mai Jiangtang capsule, α -lipoic acid, mecobalamin combined therapeutic effect of diabetic peripheral neuropathy. *Liaoning J Trad Chin Med.* (2012) 39:2227–8. doi: 10.13192/j.ljtcm.2012.11.122.mogg.086

15. Kong DM. Clinical application of Tongmai Jiangtang capsule in the treatment of diabetic peripheral neuropathy. *New World Diabetes.* (2015) 12:54–5. doi: 10.16658/j.cnki.1672-4062.2015.12.079

16. Xu J, Chen P. Analysis of therapeutic effect of Tongmai Jiangtang capsule on diabetic neuropathy. *Clin J Trad Chin Med.* (2013) 25:313–4. doi: 10.16448/j.cjtcm.2013.04.031

17. Zhang AQ, Wang HF, Li ZR. Clinical observation of tongmai jiangtang capsule in the treatment of diabetic peripheral neuropathy. *J Mod Integr Trad Western Med.* (2012) 21:1077–80.

18. Wang Q. Effect of Buchangtongmai Jiangtang capsule on diabetic peripheral neuropathy. *World Health Digest Medical Periodieal.* (2013) 26:194.

19. Chen YY, Chen Y, Zhao ZW, et al. Effect of tongmai jiangtang capsule on diabetic peripheral neuropathy. *Chin Modern Med.* (2015) 22:148–50.

20. Hao GM. Clinical efficacy observation of 30 cases of diabetic peripheral neuropathy treated with Tongmai jiangtang soft capsule combined with western medicine[J]. *World J Diabetes*. (2015) 54. doi: 10.16658/j.cnki.1672-4062.2015.06.137

21. Brok J, Thorlund K, Wetterslev J, Gluud C. Apparently conclusive Meta-analyses may be inconclusive—trial sequential analysis adjustment of random error risk due to repetitive testing of accumulating data in apparently conclusive neonatal meta-analyses. *Int J Epidemiol.* (2009) 38:287–98. doi: 10.1093/ije/dyn188

22. Xia Y, Sun Y, Liu ZL, Liu JP. Estimation of sample size in systematic review and Meta—analysis: trial sequential analysis. *J Beijing Univ Trad Chin Med.* (2013) 20:31–3. doi: 10.3969/j.issn.1672-2205.2013.05.009

23. Zippi M, Fiorino S, Budriesi R, Micucci M, Corazza I, Pica R, et al. Paradoxical relationship between proton pump inhibitors and COVID-19: a systematic review and meta-analysis. *World J Clin Cases.* (2021) 9:2763–77. doi: 10.12998/wjcc.v9.i12.2763

24. Fang ZH, Wu YL, Zhao JD. Guidelines for TCM clinical diagnosis and treatment of diabetic peripheral neuropathy (2016 edition). *Chin J Trad Chin Med.* (2017) 58:625–30. doi: 10.13288/j.11-2166/r.2017.07.025

25. Pan ML, Li S, Pan Q. Cluster analysis of TCM syndromes of diabetic peripheral neuropathy. *J Beijing Univ Trad Chin Med.* (2017) 5:394–6. doi: 10.16025/j.1674-1307.2017.05.003

26. Wang HY, Cheng Y. Research progress on Huangqi Decoction and its single herbs. *Shanghai J Tradit Chin Med.* (2015) 5:99–103. doi: 10.16305/j.1007-1334.2021.2009128

27. Liu JY, Cheng WD, Li ZH, Liu XD, Peng P, Shen S. Network-based pharmacological study on the mechanism of action of Xuan Shen in the treatment of diabetic foot. *TCM Res.* (2021) 34:50–5. doi: 10.3969/j.issn.1001-6910.2021.09.16

28. Chen X, Liang LX, Kong XQ, Ji MM, Naeem A, Li C, et al. Effect and molecular mechanism research of Astragalus membranaceus on inhibiting intestinal absorption of six alkaloids of Aconitum carmichaelii in spleen deficiency rats. *Chin Herbal Med.* (2022) 14:117–24. doi: 10.1016/j.chmed.2021.07.001

29. Hossen MJ, Amin A, Fu X-Q, Chou J-Y, Wu J-Y, Wang X-Q, et al. The anti-inflammatory effects of an ethanolic extract of the rhizome of *Atractylodes lancea*, involves Akt/NF-κB signaling pathway inhibition. *J Ethnopharmacol.* (2021) 277. doi: 10.1016/j.jep.2021.114183

30. Zhang B, Zhang L, Yu YB, Liang JF, Yang WQ, Liu J, et al. Effect of Tongmai Jiangtang capsule on the apoptosis of Schwann cells induced by AGEs and its mechanism. *Chin J Neuromed.* (2015) 14:1089–94. doi: 10.3760/cma.j.issn.1671-8925.2015.11.00

31. Babu S, Krishnan M, Rajagopal P, Periyasamy V, Veeraraghavan V, Govindan R, et al. Beta-sitosterol attenuates insulin resistance in adipose tissue via IRS-1/Akt mediated insulin signaling in high fat diet and sucrose induced type-2 diabetic rats. *Eur J Pharmacol.* (2020) 873:173004. doi: 10.1016/j.ejphar.2020.173004

32. Li F, Wang JB, Zhang SL, Zuo MH. Study on the mechanism of total glycosides of astragalus in reducing liver fibrosis in diabetic rats through PAR2 signaling pathway. *J Clin Exp Med.* (2021) 20:1043–7. doi: 10.3969/j.issn.1671-4695.2021.10.010

33. Tian HD, Wang J, Guo LN, Jia ML, Chen XP, Wang R. Study on mechanism of atractylodis rhizoma-scrophulariae radix drug pair against type 2diabetes mellitus based on network pharmacology [J]. *Chin J Mod Appl Pharm.* (2020) 165–9. doi: 10.13748/j.cnki.issn1007-7693.2020.02.008

34. Liu Q. *Exploring the Mechanism of Regulating Glycolipid Metabolism in Diabetes Mellitus Based on "Spleen Deficiency Causes Elimination" with Different Levels of Astragalus and Pueraria mirifica*. Liaoning: Liaoning University of Traditional Chinese Medicine (2020). 23–30. doi: 10.27213/d.cnki.glnzc.2020.000014

35. Duan N, Liu B, Li X, Xiong Y, Zhang Y, Zhang C, et al. Efficacy and safety of chinese patent medicine combined with oseltamivir in treatment of children with influenza: a meta-analysis. *Front Pharmacol.* (2021) 12:682732. doi: 10.3389/fphar.2021.682732

36. Liu YF, Lai BY, An T, Xiao JH, Fan YY, Pei XH. Efficacy of acupuncture for chemotherapy-induced Peripheral neuropathy: a systematic review and meta-analysis. *Shanghai J Acupunc Moxibust.* (2021) 40:511–20. doi: 10.13460/j.issn.1005-0957.2021.04.0511

37. Meng QG. Construction of evidence-based evidence system for TCM clinical efficacy evaluation. *Chin J Trad Chin Med.* (2018) 36:1031–4. doi: 10.13193/j.issn.1673-7717.2018.05.001

38. Zhang GC, Chen X, Fu WB, Jiang L, Wen XY, Xu SJ, et al. GRADE evaluation on clinical data from acupuncture treatment of anxiety disorders in china. *Chin J Trad Chin Med.* (2017) 35:1962–8. doi: 10.13193/j.issn.1673-7717.2017.08.009

39. Zhang YY, Shen C, Zhang Y, Liu JP. Misunderstanding of taking "total effective rate" as evaluation index of TCM efficacy. *Chin J Drug Eval.* (2020) 37:337–40.

40. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med.* (2013) 158:200–7. doi: 10.7326/0003-4819-158-3-201302050-00583

41. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg.* (2011). 10:28–55. doi: 10.1016/j.ijsu.2011.10.001

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 12 May 2023
DOI 10.3389/fneur.2023.1108494

OPEN ACCESS

EDITED BY
Lingyong Xiao,
First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China

REVIEWED BY
Zhonghai Yu,
Shanghai Jiao Tong University Affiliated Shanghai Sixth People's Hospital, China
Xiumin Liu,
National Institute on Drug Dependence, Health Science Centre, Peking University, China

*CORRESPONDENCE
Jun Xiang
✉ xiang.jun@zs-hospital.sh.cn
Yunke Yang
✉ yang.yunke@zs-hospital.sh.cn
Dingfang Cai
✉ cai.dingfang@zs-hospital.sh.cn

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Neurorehabilitation, a section of the journal Frontiers in Neurology

RECEIVED 26 November 2022
ACCEPTED 15 March 2023
PUBLISHED 12 May 2023

CITATION
Zhu W, Zhang W, Yang F, Cai M, Li X, Xiang Y, Xiang J, Yang Y and Cai D (2023) Role of PGC-1α mediated synaptic plasticity, mitochondrial function, and neuroinflammation in the antidepressant effect of Zi-Shui-Qing-Gan-Yin.
Front. Neurol. 14:1108494.
doi: 10.3389/fneur.2023.1108494

Role of PGC-1α mediated synaptic plasticity, mitochondrial function, and neuroinflammation in the antidepressant effect of Zi-Shui-Qing-Gan-Yin

Wen Zhu[1,2†], Wen Zhang[1,2†], Feng Yang[1,2†], Min Cai[1,2], Xiangting Li[1,2], Yijin Xiang[1,2], Jun Xiang[1,2*], Yunke Yang[1,2*] and Dingfang Cai[1,2*]

[1]Department of Integrative Medicine, Zhongshan Hospital, Fudan University, Shanghai, China, [2]Laboratory of Neurology, Institute of Integrative Medicine, Fudan University, Shanghai, China

Depression is the most prevalent psychiatric disorder, which needs deeper mechanism research studies and effective therapy. Zi-Shui-Qing-Gan-Yin (ZSQGY) is a traditional Chinese medicine decoction that has been widely used in China in the treatment of depressive symptoms. The aim of the study was to examine the anti-depressive effects of ZSQGY and the possible mechanism of action in the monosodium glutamate (MSG)-induced depressive model and the corticosterone (CORT)-induced PC12 cell model. Liquid chromatography-mass spectrometry (LC–MS) was performed to determine the major compounds in the water extract of ZSQGY. The depressive behaviors were evaluated by the field swimming test (FST), the sucrose preference test (SPT), and the open field test (OFT). Golgi staining and transmission electron microscopy (TEM) were performed to display the alterations of synaptic ultrastructure. The mitochondrion function and inflammatory factors were also quantified. The changes in peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) expression were evaluated. The results of this study demonstrated that ZSQGY significantly improved depressive behaviors. ZSQGY also reversed the changes in synaptic plasticity, improved mitochondrion function, and reduced the levels of inflammatory factors. The neuroprotective effects were accompanied by the increased expression of PGC-1α. However, the beneficial changes were reversed after the inhibition of PGC-1α. These results indicated that ZSQGY effectively could improve depressive behaviors *via* the mechanisms that regulate synaptic structural plasticity, improve mitochondrion function, and alleviate neuroinflammation, which could, or partly, attribute to the regulation of PGC-1α.

KEYWORDS

depression, Zi-Shui-Qing-Gan-Yin, synaptic plasticity, PGC-1α, neuroinflammation, mitochondrion

Introduction

Depression is a prevalent psychiatric disease with a high recurrence rate and high suicide rate, which has brought heavy health and economic burden to society (1). Approximately 350 million people worldwide suffer from depression, with a lifetime prevalence of 10–20% (2). Among them, approximately 15–25% of the patients eventually died of suicide, accounting for two-thirds of the suicide population (2). The World Health Organization estimates that by 2030, the cost of expanded treatment, mainly psychological counseling and antidepressants, will reach 147 billion dollars (3). It is indisputable that depression has become a significant global public health concern.

Although there are several antidepressant medications, only approximately half of the patients could benefit from the current options and up to 35% of patients are refractory to treatment (4, 5). Severe side effects also make these antidepressant medications far from ideal (6). It is worth noting that the antidepressants currently available are mostly based on the monoamine hypothesis of depression, which cannot explain the delay in therapeutic responses of fast-acting neurotransmitters (7, 8). The imbalance implies that adaptive mechanisms may be involved in the pathogenesis of depression. Therefore, it is necessary to explore the pathogenesis of depression and find more specific and effective therapies.

Decreased synaptic density, mitochondrial dysfunction, and activation of neuroinflammatory factors are closely related to the pathology of depression (9). Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) is a crucial co-activator that controls gene expression during mitochondrial biogenesis (10). It is also an important coordination factor that participates in the transcriptional regulation of many physiological processes, such as synaptic plasticity and neuroinflammation. The brain is the tissue with high energy demands and extremely depends on mitochondrial function, thus highlighting the crucial role of PGC-1α in the field of neurological disorders (11, 12). Subsequent studies have demonstrated that the expression of PGC-1α is attenuated in depression models and the overexpression of PGC-1α could powerfully improve the synaptic plasticity, mitochondrial function, and inflammatory response (13–15). Considering the role of PGC-1α plays in the physiological and pathological processes, there is a reasonable propose that reduced PGC-1α could contribute to the pathology of depression.

In recent years, increasing studies have reported the efficacy and safety of traditional Chinese medicine (TCM) in relieving symptoms of depression (16, 17). Zi-Shui-Qing-Gan-Yin (ZSQGY) is a TCM decoction that has been widely used in China in the treatment of depressive symptoms for a long history (18). Studies have shown that ZSQGY could alleviate the depressive symptoms of depressed patients and increase the levels of neurotransmitters in the hippocampus of depressed rats (18–21). However, little is known about the mechanisms underlying the antidepressant effects of ZSQGY. The aim of this study was to comprehensively explore the antidepressant effects and mechanism of ZSQGY in the monosodium glutamate (MSG)-induced depressive model and the corticosterone (CORT)-induced PC12 cell model.

Materials and methods

Animal

The neonate Sprague–Dawley rats (4–8 g) from Sino-British SIPPR/BK Lab Animals Ltd. (Shanghai, China) were used. The animals were kept under control conditions (12-h light/dark cycle, temperature 23 ± 1°C, relative humidity 55 ± 10%) with free access to water and food. The experiments were performed according to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Fudan University. Efforts were made to reduce the number and suffering of animals used in the study.

Monosodium glutamate (MSG)-induced depressive model

The procedure was performed as previously described (22, 23). MSG (Sigma-Aldrich, St. Louis, MO, United States) was dissolved in 0.9% NaCl solution to the final concentration of 10%. Briefly, the experimental animals were subcutaneously injected with MSG at a dose of 4 mg/g on the postnatal 2nd, 4th, 6th, 8th, and 10th days. The control animals received an equimolar concentration of sodium chloride. The animals were weaned on the 21st day, and the female rats were removed from the cage.

ZSQGY preparation and quality control

ZSQGY consists of 12 crude drugs including Radix Rehmanniae, Dioscoreae Rhizoma, Radix Bupleuri, Radix Paeoniae Alba, Radix Angelicae Sinensi, Rhizoma Anemarrhenae, Fructus Corni, Cortex Moutan, Poria, Ziziphi Spinosae Semen, Rhizoma Alismatis, and Gardeniae Fructus. The composition of ZSQGY is listed in Table 1. All crude drugs were obtained from Zhongshan Hospital. Briefly, the crude herbs were boiled in 10 times the volume of drinking water for 1 h for three times. Suspensions filtered from three decoctions were mixed and centrifuged at 2,000 *g* for 20 min. The collected suspension was soaked in 100% ethanol under rapid agitation, followed by stirring overnight. Then the suspension was collected and centrifuged at 2,000 *g* for 20 min and concentrated to 2 g/mL (w/v) before autoclaving.

LC–MS analysis of ZSQGY

Liquid chromatography-mass spectrometry (LC–MS) was established for the qualitative analysis of chemical compounds of ZSQGY. The API 4000 tandem mass spectrometer (Applied Biosystems/MDS SCIEX, United States) equipped with an electrospray ionization source (ESI) was used in the LC–MS analysis. All targeted analyses were performed in positive and negative ion modes. The curtain gas (CUR) and collision gas (CAD) used high-purity (99.99%) nitrogen. The ionspray voltage (IS) was set 3,500 V and the ionspray temperature (TEM) was 450°C. The 100 μL samples were dissolved in a 1 mL solution containing methanol and filtered through a 0.22 μm membrane. The content of the components in ZSQGY was calculated using the point external standard method.

TABLE 1 Composition of ZSQGY.

Ingredients (Latin name)	Ingredients (Chinese name)	Family	Part used	Ratio (g)
Radix Rehmanniae	Di Huang	Scrophulariaceae	Root	12
Dioscoreae Rhizoma	Shan Yao	Dioscoreaceae	Root and rhizome	12
Radix Bupleuri	Chai Hu	Umbelliferae	Root	10
Radix Paeoniae Alba	Bai Shao	Ranunculaceae	Root	10
Radix Angelicae Sinensi	Dang Gui	Umbelliferae	Root	10
Rhizoma Anemarrhenae	Zhi Mu	Liliaceae	Root and rhizome	12
Fructus Corni	Shan Zhu Yu	Cornaceae	Flesh	12
Cortex Moutan	Mu Dan Pi	Ranuculaceae	Velamen	10
Poria	Fu Ling	Polyporaceae	Sclerotium	10
Ziziphi Spinosae Semen	Suan Zao Ren	Rhamnaceae	Seed	15
Rhizoma Alismatis	Ze Xie	Alismatales	Stem	10
Gardeniae Fructus	Zhi Zi	Rubiaceae	Seed	10

Experimental groups of animals and drug administration

The animals (8 weeks old) were randomly divided into the control group, the model group, the L-ZSQGY group, the M-ZSQGY group, the H-ZSQGY group, and the FXT group. The dose of ZSQGY that translated from human beings to animals in this study was established using the body surface area normalization method. For ZSQGY treatment, the common human daily dose in the present study is 133 g/70 kg bodyweight. According to the formula drat = human × 0.71/0.11, we selected 12, 24, and 48 g/kg/day as low, middle, and high doses, respectively. For fluoxetine treatment, the daily dose required for an adult is 20 mg/70 kg, which is converted to 0.184 mg/100 g for a rat. The treatment dosage for rats was 6.45 times that of patients. The animals in the control group and mode group were taken in the same volume as that of normal saline. All drugs were given once daily continuously for 4 weeks.

Forced swimming test (FST)

The rats were placed in an acrylic cylinder (height 40 cm, and diameter 20 cm) filled with water (depth 20 cm) at a temperature of 25 ± 1°C for 15 min (the pretest session). After 24 h, the rats were forced to swim again for 5 min. The duration of immobility (s) was analyzed. The rats remained floating in an upright position, with small movements of the four limbs to keep its head above the water surface, and were judged to be immobile.

Sucrose preference test (SPT)

Briefly, the rats were presented with two bottles containing 1% sucrose solution in the home cage for 24 h. Then, either water or 1% sucrose solution was placed in a random order in the home cage for another 24 h and the bottle order was exchanged every 12 h to account for side preference. The animals were deprived of water for 12 h before the test and then presented with two pre-weighed bottles containing 1% sucrose solution or water. The intake of water, sucrose solution, and total fluid intake were measured after 18 h. The percentage of sucrose solution intake in relation to the total fluid intake was calculated. The impaired sucrose intake was indicative of the depression-like behavior.

Open field test (OFT)

Central and peripheral areas were divided in the open field apparatus. The animal was placed individually in the open field area (50 × 50 × 50 cm) for 5 min, and the locomotor activity was videotaped (Ethovision 9.0, Noldus). Time spent in center (s) and latency to center (s) were analyzed.

Golgi staining

Golgi staining and spine density analysis were performed according to the manufacturer's instructions (Hito Golgi-Cox OptimStain kit, Hitobiotec Corp, Kingsport, TN, United States). The medial prefrontal cortex (mPFC) was cut into 80 μm thickness coronal tissue sections using a freezing microtome (Microm HM 450, Waldorf, Germany). Neurons in the mPFC area were analyzed. Dendritic spine density was counted by randomly selecting the secondary and tertiary apical dendrites and was shown as the number of thorns/10 μm dendrite. In total, three segments were counted per section, and three slides were chosen from each rat.

Transmission electron microscopy (TEM)

The rats were given anesthesia with sodium pentobarbital (40 mg/kg) intraperitoneal injection, and the mPFC were removed and incubated in 2.5% glutaraldehyde at 4°C for 24 h. Then, the mPFC were cut into 1 mm^3 segments and fixed in 1% osmium tetroxide for 2 h. The tissue segments were rinsed and dehydrated in a series of graded aqueous ethanol and embedded in Epon. A

total of 70 nm ultrathin sections were prepared and stained with 3% uranyl acetate and 0.5% lead citrate. Images were taken using the TEM (6,200× magnification) and analyzed using Image Pro Plus.

Immunohistochemistry (IHC)

The rats were anesthetized with sodium pentobarbital (40 mg/kg) intraperitoneal injection and then transcardially perfused with 4% paraformaldehyde. The brains were removed and then paraffin was embedded. Then the mPFC slides (10 μm) were incubated in PBS containing 0.03% H_2O_2 for 10 min and in 5% goat serum for 30 min, followed by the incubation with anti-PGC-1α (1:100; Abcam, Cambridge, United Kingdom) at 4°C overnight. After washing with PBS, the sections were incubated with a secondary antibody (1:200) for 1 h. Then slices were added an appropriate amount of horseradish enzyme and incubated at 37°C for 30 min. After washing with PBS, the slides were incubated in DAB. The nuclei were stained with hematoxylin. The slices were dehydrated with ethanol, sealed with gum, and further observed under the microscope for observation and analysis (Olympus BX51, Tokyo, Japan).

qRT-PCR

The total RNA was extracted using a Trizol Reagent (Introgen, Carlsbad, CA, United States) according to the instructions. The total RNA was reverse transcribed into cDNA using the cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, United States). A real-time quantitative PCR analyzer was used to detect the expressions of PGC-1α mRNA and PSD95 mRNA. The obtained genes were normalized to GAPDH. The primers sequences were listed as follows: GAPDH, AACTCCCATTCCTCCACCTT, and GAGGGCCTCT CTCTTGCTCT; PGC-1α, AGGCAAGCAAGCAGGTCT, and GTCATCAAACAGGCCATCC; and PSD95, GCAGGTTG CAGATCGGAGAC, and CCAGGTGCTGAGAATATGAGGTT.

Measurement of mitochondrial DNA (mtDNA)

The analysis of the mtDNA amount was measured by quantitative PCR. The copy numbers of mtDNA were normalized to a nuclear-encoded gene RBM15.

Enzyme-linked immunosorbent assay

The concentrations of malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-1 (IL-1), 6 (IL-6) from the mPFC of rats, and cell serum were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology, Shanghai, China).

Measurement of mitochondrial ATP level

The mitochondrial ATP level was detected using the ATP Assay kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions.

Preparation of the ZSQGY-containing serum

The rats were administered with the ZSQGY (24 g/kg/day) for 3 consecutive days. After anesthetized with sodium pentobarbital (40 mg/kg), the abdominal aorta blood of the animals was collected 2 h after the treatments and centrifuged at 4°C for 20 min, followed by sterilization through a 0.22 μm microporous membrane. The serum was stored at −80°C.

Cell culture and drug treatment

High concentrations of CORT can induce the neurotoxicity of PC12 cells, which has been widely used as an *in vitro* model for depression. In this study, differentiated PC12 cells were maintained (at a density of 1 × 105 cells/mL) in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin–streptomycin at 37°C under a humidified atmosphere of 5% CO_2 and 95% air (v/v) for 24 h. Plated PC12 cells were exposed to CORT (Sigma-Aldrich, St. Louis, MO, United States) at the level of 200 and 400 μM for 24 h, and then the PC12 cells were treated with different concentrations of the ZSQGY-containing serum (5, 10, and 20%) to determine the suitable doses of CORT and ZSQGY-containing serum by assessing with the CCK-8 kit (Beyotime Biotechnology, Shanghai, China).

Viability assay

PC12 cells were treated with the CCK-8 solution and incubated at 37°C for 4 h. The optimal density at 450 nm was measured with a microplate reader (Bio-Rad Laboratories, Hercules, CA, United States). Cell viability was presented as a percentage of control cells.

Grouping of PC12 cells and PGC-1α siRNA transfection

The PC12 cells were divided into the control group, the model group, the ZSQGY + PGC-1α siRNA group, and the ZSQGY + con-siRNA group. For the control group, PC12 cells were cultured under the abovementioned normal conditions. For the model group, PC12 cells were exposed to 200 μM CORT for 24 h. For the ZSQGY + PGC-1α siRNA group, PC12 cells were treated with PGC-1α siRNA before modeling and ZSQGY-containing serum treatment. For the ZSQGY + con-siRNA group, PC12 cells were treated with con-siRNA before modeling and ZSQGY-containing serum treatment. The PGC-1α siRNA and con-siRNA (Jikai Biology, Shanghai, China) were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, United States).

Statistical analysis

Data are expressed as mean ± standard error. Data were analyzed by one-way analysis of variance (ANOVA) followed by the LSD multiple comparison test using software SPSS 20.0. Data were considered statistically significant at a value of p of less than 0.05.

Results

Qualitative and quantitative analysis of components in water extract of ZSQGY

Qualitative analysis of compounds in water extract of ZSQGY was carried out by LC–MS. A total of six compounds, including geniposide, paeoniflorin, albiflorin, ferulic acid, saikosaponin A, and ursolic acid in water extract were used for quantitative analysis (Figure 1). After calculation, this water extract contained 533.682 μg/mL of geniposide, 474.789 μg/mL of paeoniflorin, 225.892 μg/mL of albiflorin, 280.147 μg/mL of ferulic acid, 30.411 μg/mL of saikosaponin A, and 11.468 μg/mL of ursolic acid, respectively (Table 2).

ZSQGY improved the depressive behaviors in the MSG-induced depressive model

The forced swimming test, the sucrose preference test, and the open field test were administrated to evaluate the effects of ZSQGY against depressive behaviors. In the forced swimming test, the rats in the model group spent longer immobility time during the test ($p < 0.05$; Figure 2A). The rats in the ZSQGY group and fluoxetine group spent shorter immobility time than the rats in the model group ($p < 0.05$; Figure 2A). In the sucrose preference test, we observed that compared with the control group, the consumption of sucrose in the model group decreased ($p < 0.05$; Figure 2B). However, the consumption of sucrose in rats that received ZSQGY or fluoxetine treatment increased significantly ($p < 0.05$; Figure 2B). In the open field test, the rats in the model group spent less time in the central area than those in the control group ($p < 0.05$; Figures 2C–E). The rats in the model group also traveled a shorter distance than those in the control group. In contrast, the rats who received ZSQGY or fluoxetine treatment spent a longer time in the central area and traveled a longer distance ($p < 0.05$; Figures 2C–E).

ZSQGY ameliorated impaired synaptic structural plasticity in mPFC in the MSG-induced depressive model

Results from the Golgi staining showed that compared with the control group, the density of dendritic spines in mPFC in the MSG group induced decreased significantly ($p < 0.05$; Figures 3A,B). The treatments of ZSQGY and FXT significantly reversed a decrease in dendritic spine density induced by MSG ($p < 0.05$; Figures 3A,B). To further corroborate the results from Golgi staining, the mPFC neuronal ultrastructure was examined by TEM. The PSD thickness in asymmetric synapses in the model group was significantly thinner than those in the control group ($p < 0.05$, Figures 3C,E). However, the treatments of ZSQGY and FXT remarkably ameliorated the reduction of the PSD thickness ($p < 0.05$, Figures 3C,E). In addition, the number

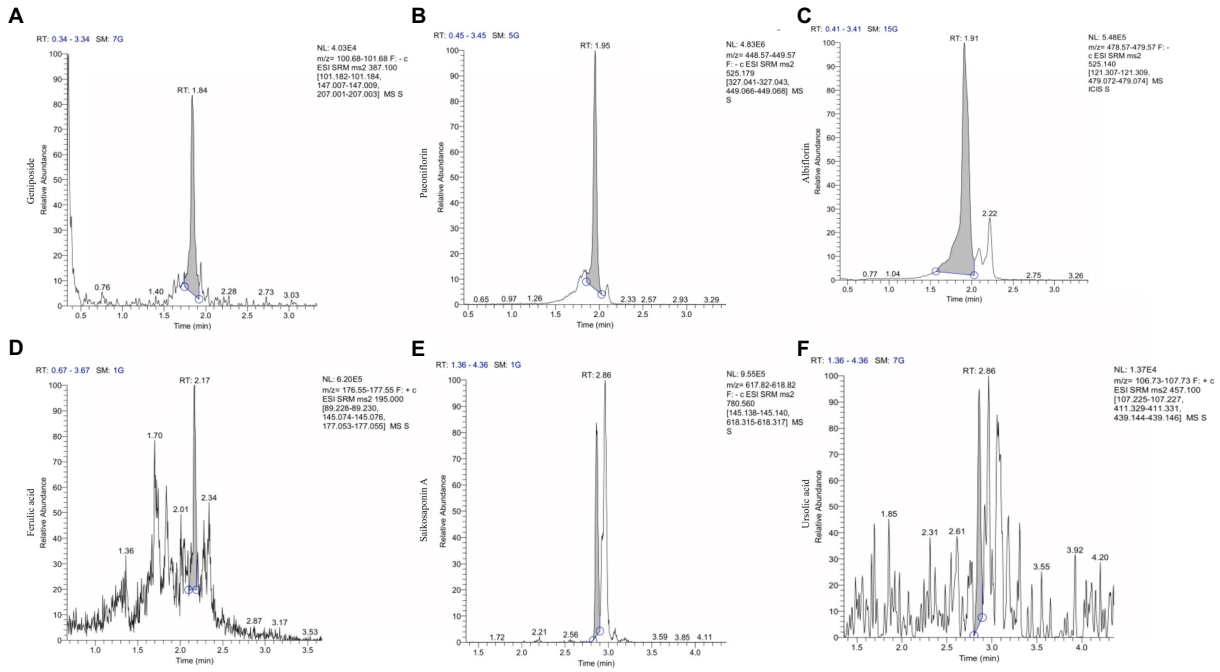


FIGURE 1
The ion chromatograms (TICs) of geniposide, paeoniflorin, albiflorin, ferulic acid, saikosaponin A and ursolic acid in ZSQGY. **(A)** Geniposide; **(B)** Paeoniflorin; **(C)** Albiflorin; **(D)** Ferulic acid; **(E)** Aaikosaponin A; **(F)** Fursolic acid.

10.3389/fneur.2023.1108494

TABLE 2 Quantitative analysis of six major compounds in ZSQGY water extract analyzed by LC–MS.

Chemical ingredients	Liner range (μg/mL)	Regression equation	Correlation coefficient®	Actual concentration (μg/mL)
Geniposide	1–100	$Y = 3221.15 + 9301.68x$	0.9933	533.682
Paeoniflorin	0.1–50	$Y = -28193.1 + 1.18247e{+}006x$	0.9922	474.789
Albiflorin	0.01–100	$Y = -2638.02 + 65{,}849x$	0.9953	225.892
Ferulic acid	1–100	$Y = -69442.4 + 193{,}791x$	0.9922	280.147
Saikosaponin A	0.01–10	$Y = 1507.57 + 2.39811e{+}006x$	0.9911	30.411
Ursolic acid	0.1–10	$Y = -2287.96 + 136{,}156x$	0.9993	11.468

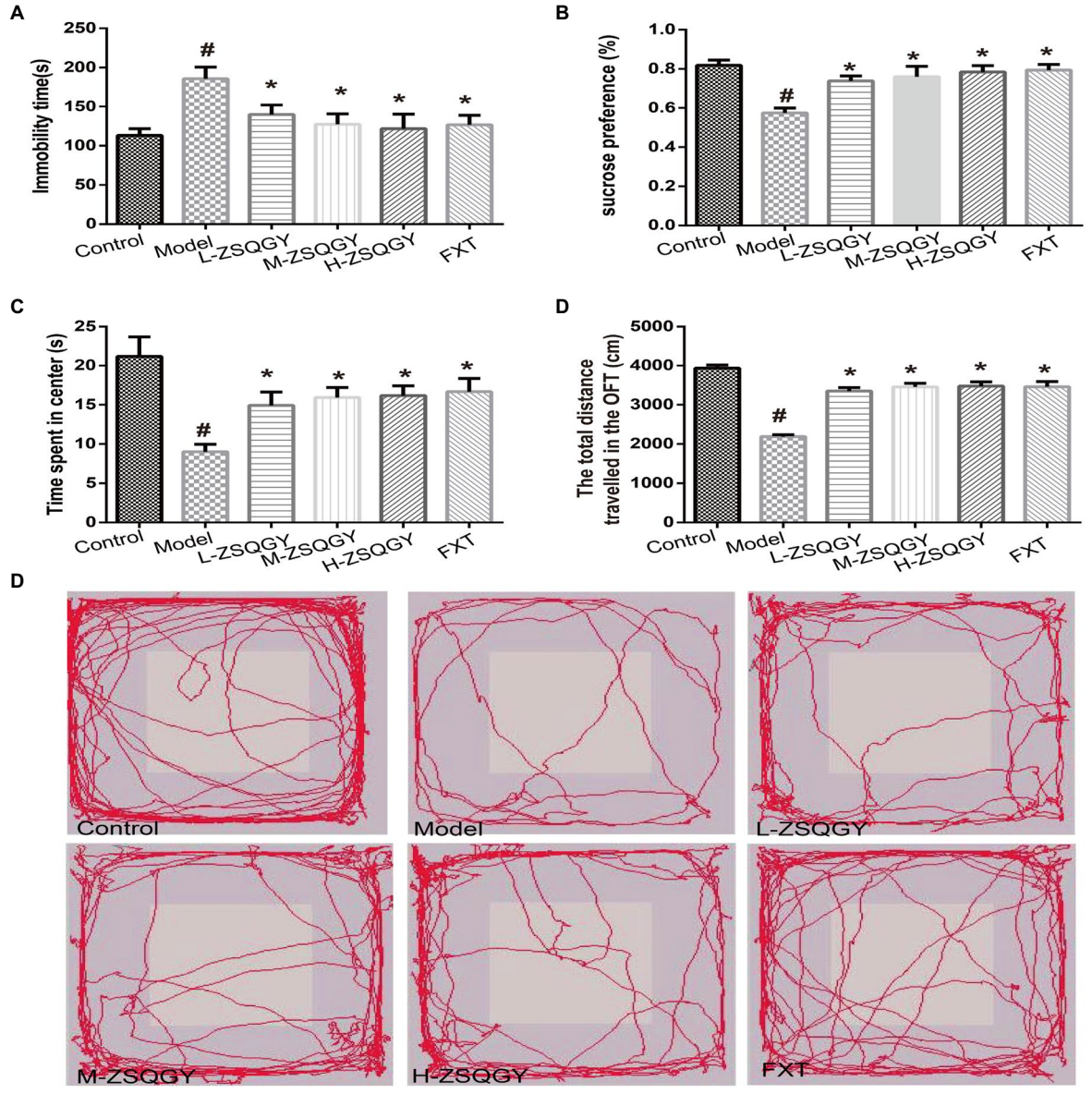


FIGURE 2
ZSQGY improved depressive behaviors in the MSG-induced depressive model. **(A)** Immobility time in the forced swimming test. **(B)** Sucrose preference (%) in the sucrose preference test. **(C)** Time spent in center (s) in the open field test. **(D)** Total distance traveled in the open field test. **(E)** Trace plot of the open field test. Data are presented as mean±SE. #p<0.05 vs. control group. *p<0.05 vs. model group (n=12 in each group).

10.3389/fneur.2023.1108494

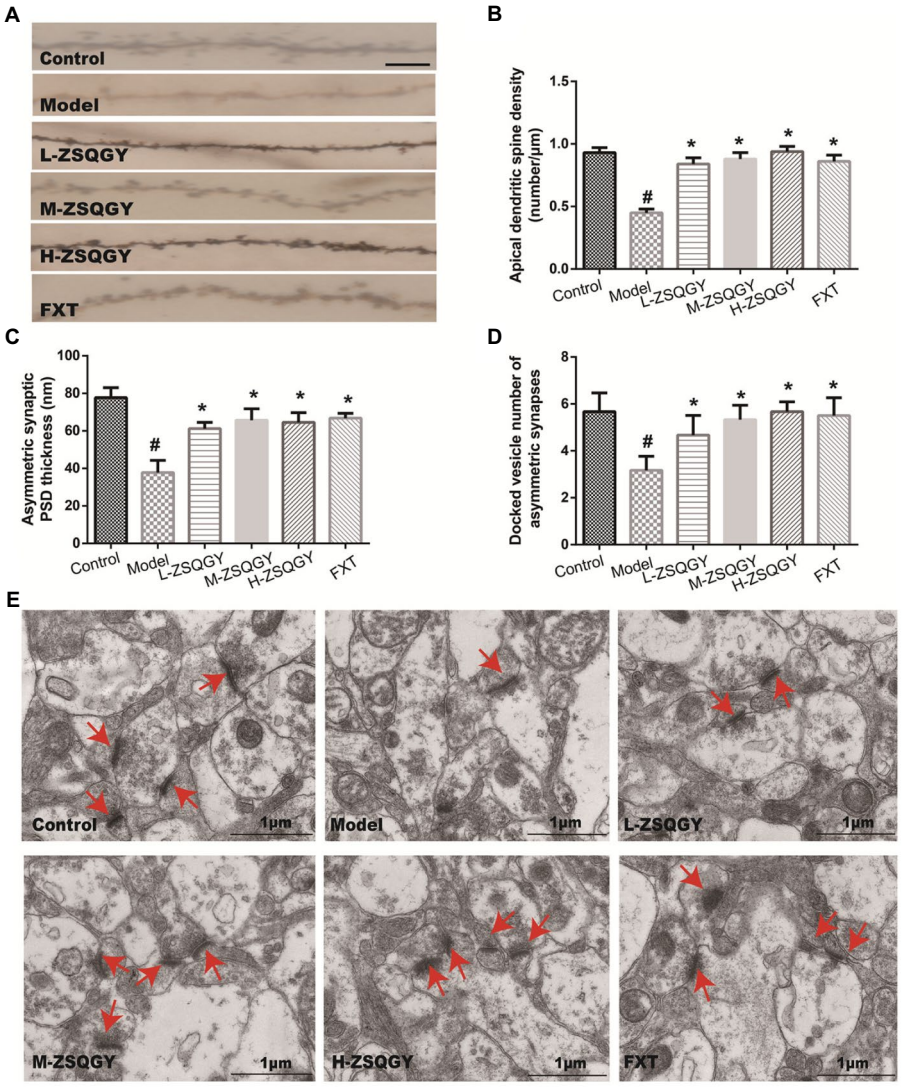


FIGURE 3
ZSQGY ameliorated impaired synaptic structural plasticity in mPFC in the MSG-induced depressive model. **(A)** Golgi staining of the apical dendritic spine (scale bar, 5μm). **(B)** Apical dendritic spine density (number/μm). **(C)** Asymmetric synaptic PSD thickness (nm). **(D)** Docked vesicle number of asymmetric synapses. **(E)** Ultrastructure of asymmetric synapses in the mPFC (scale bar, 1μm). Data are presented as mean±SE. #p<0.05 vs. control group. *p<0.05 vs. model group (n=6 in each group).

of docked vesicles in asymmetric synapses was decreased in the model group ($p < 0.05$, Figures 3D,E). However, the reduction was reversed by the treatment of ZSQGY or FXT ($p < 0.05$, Figures 3D,E).

ZSQGY attenuated the damaged mitochondrial function in the MSG-induced depressive model

As shown in Figure 4, the levels of 8-OHdG and MDA in the model group were higher when compared with those in the control group ($p < 0.05$; Figures 4A,B). Contrarily, the levels of 8-OHdG and MDA decreased in rats that received the treatment of ZSQGY or FXT ($p < 0.05$; Figures 4A,B). In addition, the ATP content and mtDNA copy number of the model group were significantly decreased when compared with those in the control group ($p < 0.05$; Figures 4C,D). The application of ZSQGY eminently counteracted the decrease of ATP content and mtDNA copy number ($p < 0.05$; Figures 4C,D).

ZSQGY attenuated the level of inflammatory cytokines in the MSG-induced depressive model

As shown in Figure 5, the levels of IL-1β, IL-6, TNF-α, and IFN-γ were elevated in the model group compared to those in the control group ($p < 0.05$; Figures 5A–D). Notably, ZSQGY and FXT reduce the content of inflammatory cytokines induced by MSG ($p < 0.05$; Figures 5A–D).

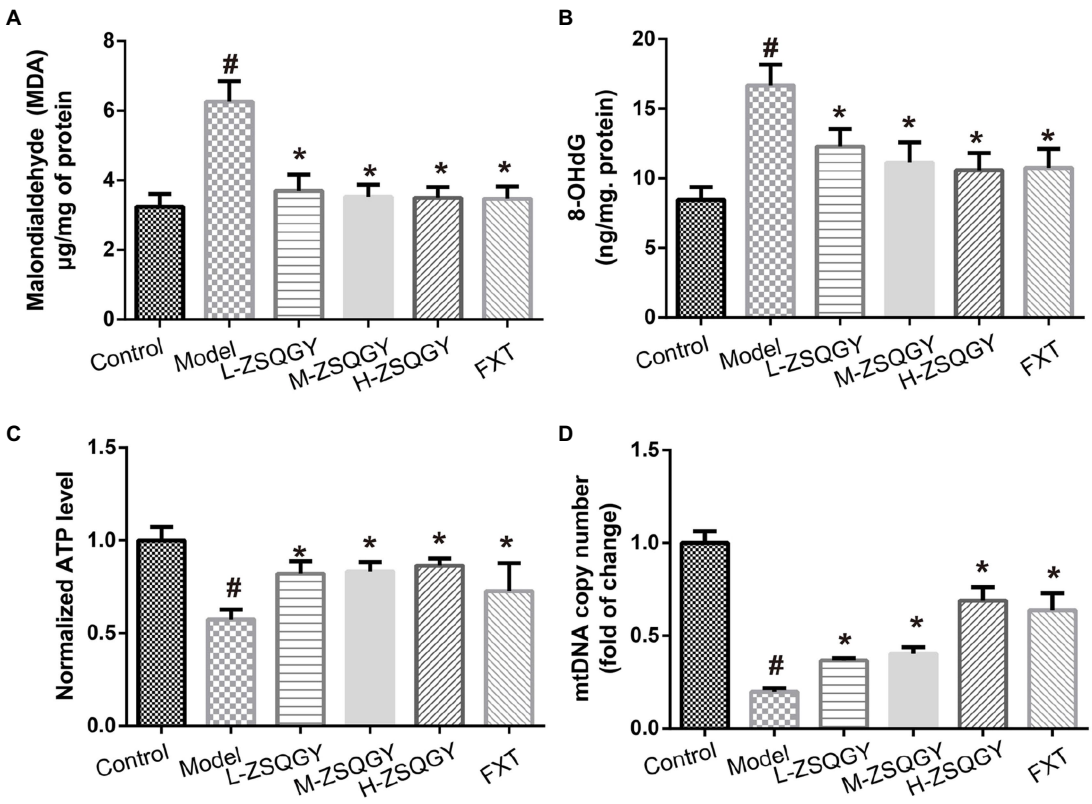


FIGURE 4
ZSQGY attenuated the damage of mitochondrial function in the MSG-induced depressive model. **(A)** ELISA of MDA. **(B)** ELISA of 8-OHdG. **(C)** ATP content. **(D)** mtDNA copy number. Data are presented as mean±SE. #p<0.05 vs. control group. *p<0.05 vs. model group (n=6 in each group).

ZSQGY attenuated the reduction of PGC-1α in the MSG-induced depressive model

As shown in Figure 6, the level of PGC-1α mRNA significantly decreased in the model group compared with those in the control group ($p < 0.05$; Figure 6A). However, the level of PGC-1α mRNA significantly increased in the rats that received ZSQGY or FXT ($p < 0.05$; Figure 6A). The results of IHC showed that PGC-1α is stained brownish yellow or brown and expressed in both the nucleus and cytoplasm. In comparison with the control group, the mean optical density of PGC-1α was significantly decreased ($p < 0.05$; Figures 6A,B). In contrast, the mean optical density of PGC-1α was increased remarkably in the ZSQGY group and in the FXT group ($p < 0.05$; Figures 6A,B).

ZSQGY protects PC12 cells against CORT-induced injury

To screen out the appropriate concentration of CORT to damage PC12 cells as well as the cytoprotective effects of ZSQGY against CORT-induced injury, the PC12 cells were treated with high concentrations of CORT (200 and 400 μM) for 24 h, followed by treatment with different concentrations of ZSQGY-containing serum (5, 10, and 20%) for 24 h. As shown in Figure 7A, 200 μM CORT treatment caused a decrease of approximately 50% of viable cells. In addition, 10% ZSQGY-containing serum significantly increased cell viability, while there were no significant changes in the viability of cells in PC-12 cells treated with 5 and 20% ZSQGY-containing serum. Therefore, 200 μM CORT and 10% ZSQGY-containing serum were selected for the following studies.

To further explore whether PGC-1α is implicated in the beneficial effects of ZSQGY, the PGC-1α siRNA was used to inhibit its functions and then the levels of postsynaptic density 95 (PSD95), oxidative stress markers, and inflammatory cytokines were determined. As shown in Figures 7B–J, compared to the control group, the levels of oxidative stress markers (8-OHdG, MDA) and inflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) were significantly increased in the CORT-induced PC12 cells, while the levels of PSD95 mRNA, ATP, and mtDNA were decreased in the CORT-induced PC12 cells ($p < 0.05$; Figures 7B–J). The ZSQGY-containing serum treatment notably decreased the levels of 8-OHdG, MDA, and inflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) ($p < 0.05$; Figures 7B–J). This treatment also significantly increased the levels of PSD95 mRNA, ATP, and mtDNA ($p < 0.05$; Figures 7B–J). However, the beneficial effects of ZSQGY in synaptic plasticity, mitochondrial function, and neuroinflammation were blocked after the administration of PGC-1α siRNA ($p < 0.05$; Figures 7B–J).

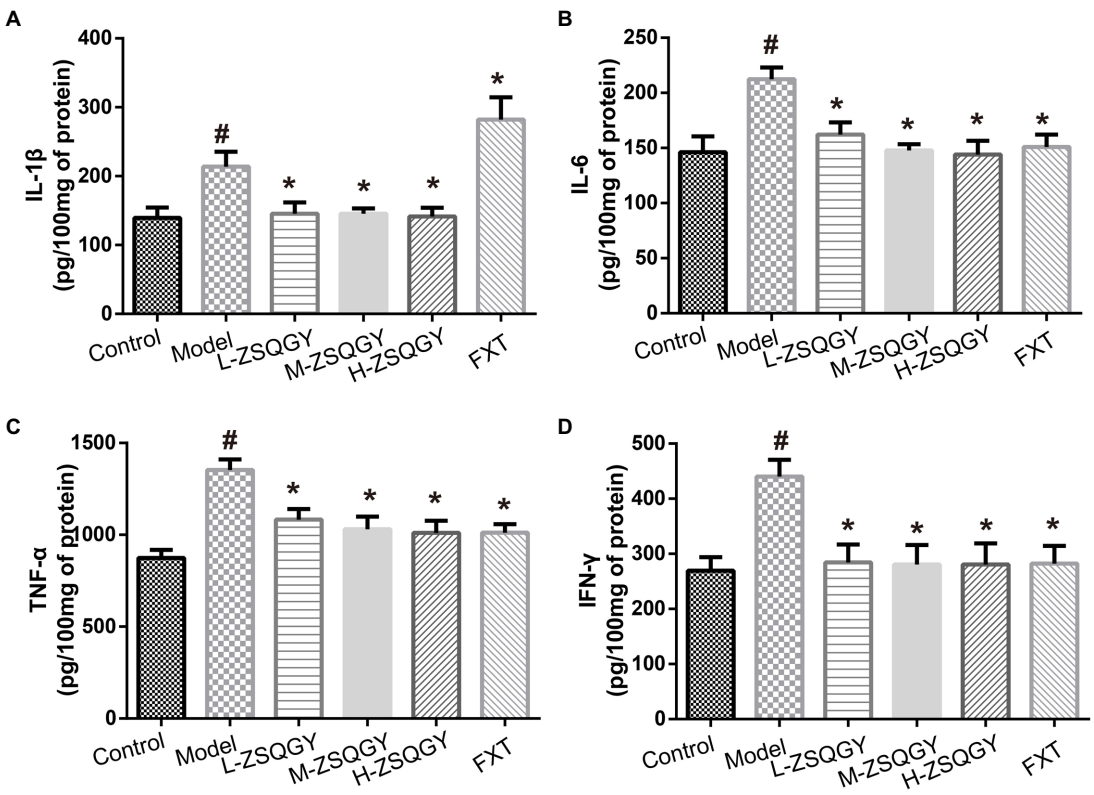


FIGURE 5
ZSQGY attenuated the level of inflammatory cytokines. **(A)** Level of IL-1β. **(B)** Level of IL-6. **(C)** Level of TNF-α. **(D)** Level of IFN-γ. Data are presented as mean±SE. #p<0.05 vs. control group. *p<0.05 vs. model group (n=6 in each group).

Discussion

In this study, we evaluated the antidepressant effects and mechanism of ZSQGY. The results showed that ZSQGY could improve depressive behaviors in the MSG-induced depressive model. In addition, ZSQGY significantly improved the damaged synaptic structural plasticity, attenuated the dysfunction of the mitochondrion, and reduced the level of inflammatory cytokines in *in vivo* and *in vitro* depression models. However, the beneficial changes were reversed after the inhibition of PGC-1α.

One of the important pathological features of patients with depression is the damaged synaptic density, which could lead to the interruption of neural circuits and the abnormality of brain structure caused (24, 25). Studies have reported that a reduced size of the prefrontal cortex (PFC) and decreased neuronal synapses are associated with depression (26). The dendritic spine is the morphological specialization that protrudes from the dendritic shaft. Structural changes in the dendritic spine are considered the basis of synaptic plasticity. Converging evidence from clinical and experimental studies indicates that the impaired dendritic spine is involved in the pathology of multiple diseases (27). The loss of dendritic spines is accompanied by depression-like behaviors, suggesting that the structural changes and neuronal atrophy in mPFC are associated with depression (28–30). Moreover, synaptic ultrastructural alterations, such as decreased PSD thickness, are associated with depressive behavioral changes (31, 32). In this study, ZSQGY remarkably increased the dendritic spine density, the PSD thickness, and the number of docked vesicles, indicating that ZSQGY administration could improve the damaged synaptic structural plasticity.

The energy metabolism disorder caused by mitochondrial dysfunction contributes to the pathogenesis of depression (33). As the energy factory of cells, mitochondria participates in maintaining calcium homeostasis and regulating the generation of reactive oxygen species (ROS) and cell apoptosis (34). Moreover, mitochondria in the central nervous system can enable synaptic plasticity and promote neural differentiation and neurotransmitter release (35). Internal and external cues can lead to the reduction of ATP synthesis and excessive production of ROS, which may affect behaviors by interfering with synaptic plasticity. In addition, internal and external stimuli could induce damage to mtDNA and mitochondrial dysfunction (36). Patients with severe depression usually demonstrate mitochondrial energy metabolism disorder, such as a lower level of ATP and a higher level of ROS, as well as the reduction of mitochondrial copy number (37). The results of our study suggested ZSQGY could attenuate the level of 8-OHdG and MDA, which are markers of oxidative stress during mitochondrial disturbance. In addition, ZSQGY could improve the decrease of ATP content and mtDNA copy number in the MSG-induced depression model. These findings indicate that ZSQGY could restore mitochondrial function.

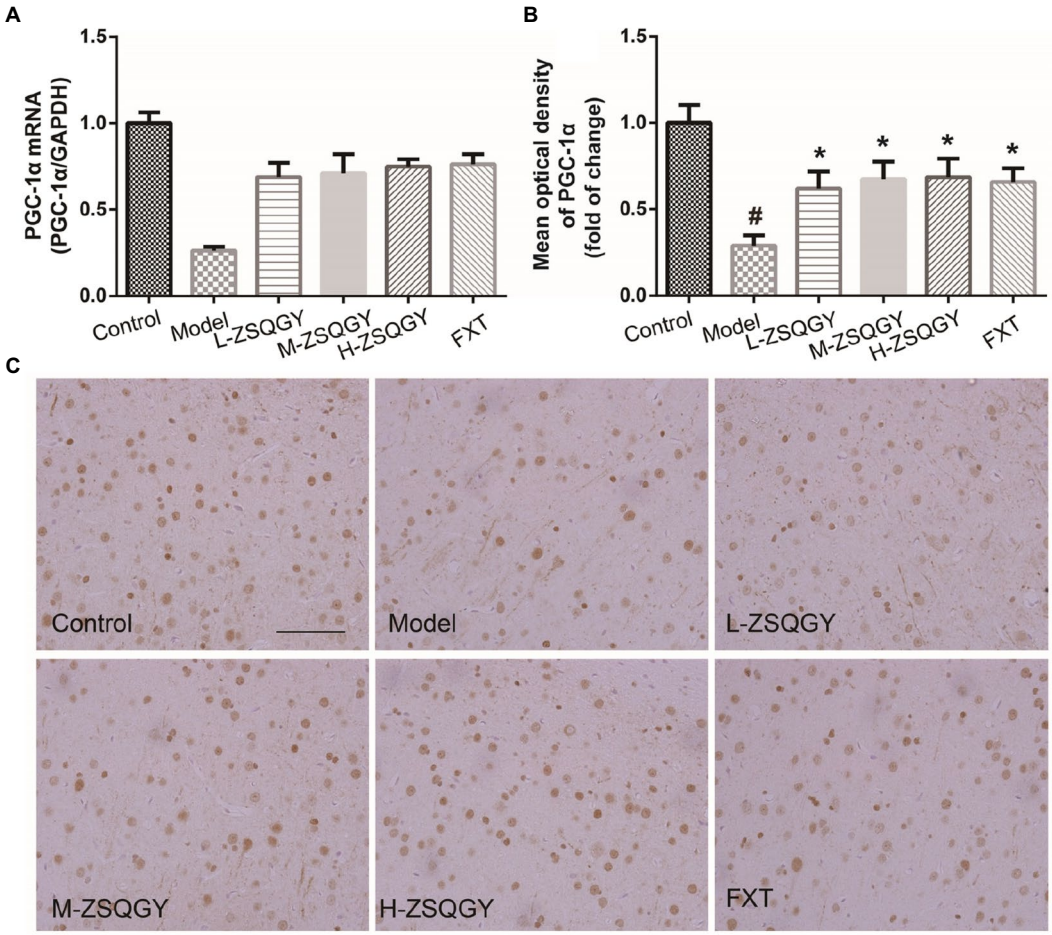


FIGURE 6
ZSQGY attenuated the reduction of PGC-1α in the MSG-induced depressive model. **(A)** Level of PGC-1α mRNA. **(B)** Mean optical density of PGC-1α. **(C)** IHC of PGC-1α. Data are presented as mean±SE. #p<0.05 vs. control group. *p<0.05 vs. model group (n=6 in each group).

It is well established that patients with depression exhibit increased circulating levels of inflammatory factors (38, 39). Inflammatory cytokines are key regulators of neuronal functions, and they play an important role in maintaining synaptic plasticity (40). Long-term exposure to psychosocial pressure disturbs the inflammatory cascade, leading to excess or prolonged inflammatory cytokines that interfere with synaptic plasticity and eventually leading to depressive symptoms (41, 42). Excessive ROS production and mtDNA damage induced by stress also trigger an inflammation response (43). Notably, ZSQGY was able to reduce the levels of IL-1β, IL-6, TNF-α, and IFN-γ, which demonstrated the anti-inflammatory effects of ZSQGY in the central nervous system in the MSG-induced depression model.

PGC-1α is a well-known ligand-activated transcription co-activator that mainly expresses in high energy demand tissues, e.g., brain, heart, and kidney (44). Once activated by different stimuli, PGC-1α translocates from the cytoplasm to the nucleus, where it interacts with nuclear respiratory factor 1 (NRF-1) and NRF-2 (45). As a result, mitochondrial transcription factor A (TFAM) is activated, increasing the expression of nuclear genes and further promoting mtDNA replication and transcription (46). It has been reported that PGC-1α deficiency could influence oxidative metabolism, then leading to axonal degeneration in the brain. PGC-1α also participates in the procedure of synaptogenesis. PGC-1α can enhance the expression of synaptic proteins, such as PSD95 (47, 48). In addition, PGC-1α is engaged in the process of macrophage polarization from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype and then participates in the inflammation responses (49, 50). In this study, we found that ZSQGY could increase the expression of PGC-1α, accompanied by improvements in synaptic plasticity, mitochondrial function, and inflammation responses. Our findings are in line with the previous studies in which upregulating the level of PGC-1α could improve depression-like behaviors (51, 52). Moreover, the beneficial effects of ZSQGY were reversed after the administration of PGC-1α siRNA in the CORT-induced PC12 cell model. These results indicated that ZSQGY effectively could improve depressive behaviors *via* the mechanisms that regulate

10.3389/fneur.2023.1108494

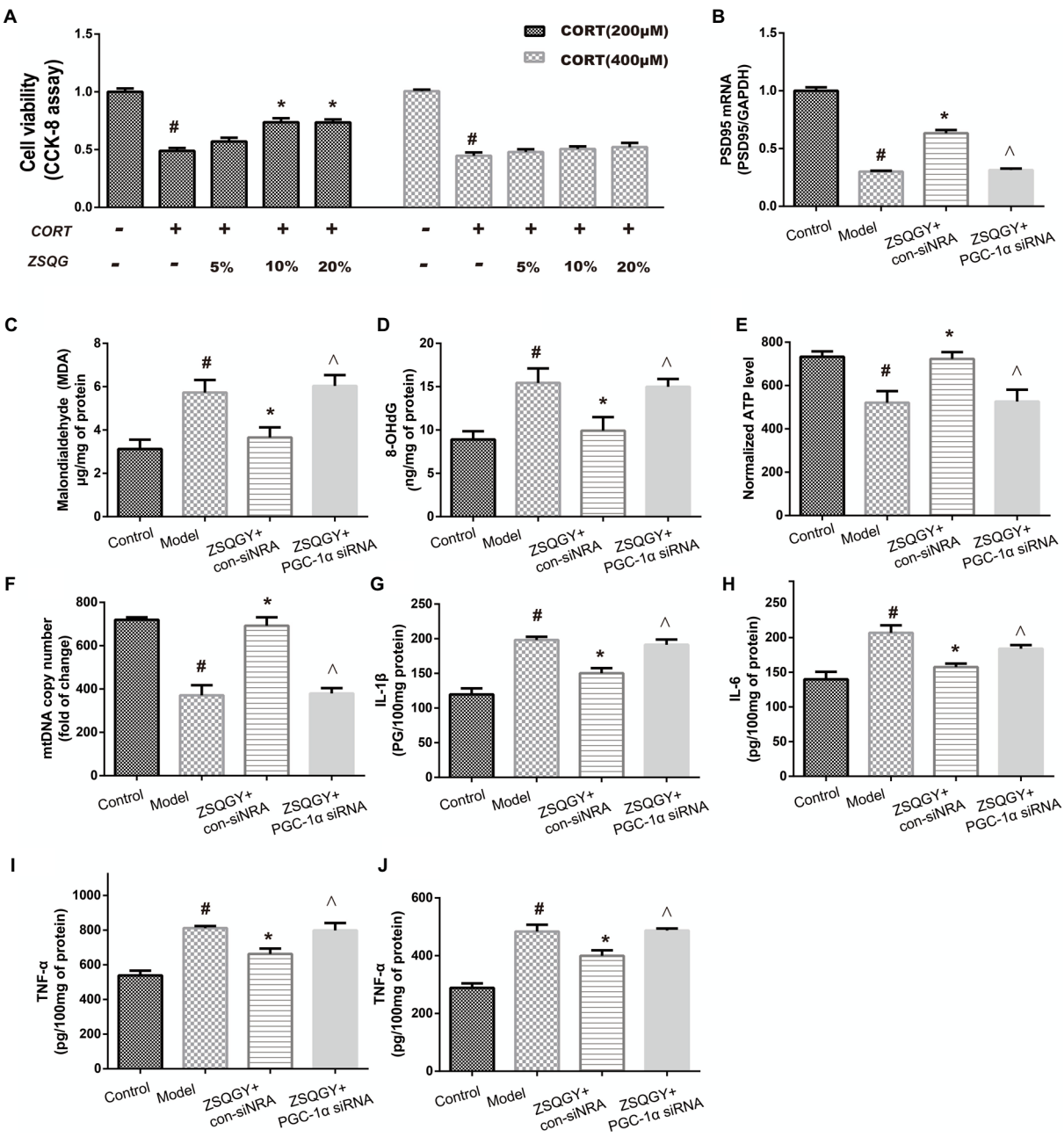


FIGURE 7
ZSQGY protects PC12 cells against CORT-induced injury. **(A)** Cell activity measured CCK8 assay. **(B)** Level of PSD95 mRNA. **(C)** ELISA of MDA. **(D)** ELISA of 8-OHdG. **(E)** ATP content. **(F)** mtDNA copy number. **(G)** Level of IL-1β. **(H)** Level of IL-6. **(I)** Level of TNF-α. **(J)** Level of IFN-γ. Data are presented as mean±SE. #p<0.05 vs. control group. *p<0.05 vs. model group. ^p<0.05 vs. ZSQGY+con-siRNA group (n=6 in each group).

synaptic structural plasticity, improve mitochondrion function and alleviate neuroinflammation, which could, or partly, attribute to the regulation of PGC-1α.

The water extract of ZSQGY contains many bioactive compounds, however, in which chemical ingredients responsible for the beneficial effects of ZSQGY remain unknown. Here, we detected six major compounds of ZSQGY, including saikosaponin A, ferulic acid, albiflorin, paeoniflorin, geniposide, and ursolic acid. Previous studies have demonstrated that these chemical ingredients displayed anti-depressive effects in models of depression and the therapeutic mechanisms underlying these ingredients involve neuroendocrine, neuroinflammation, and neurotrophic systems (53). These findings provided evidence for the therapeutic effects of ZSQGY. In future, more studies are needed to discover the mechanisms that are underlying the synergistic antidepressant effects of these chemical ingredients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Ethics Committee of Zhongshan Hospital, Fudan University.

Author contributions

WZhu and FY contributed to study design, data interpretation, and revision of the manuscript. WZha contributed to revision of the manuscript. MC and YX contributed to data interpretation. JX and XL contributed to statistical analysis and data interpretation. YY and DC provided final approval to submit the manuscript for publication. All authors reviewed the manuscript and approved the final manuscript.

Funding

This work was supported by the Development Project of Shanghai Peak Disciplines-Integrative Medicine (No. 20180101).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Burcusa SL, Iacono WG. Risk for recurrence in depression. *Clin Psychol Rev*. (2007) 27:959–85. doi: 10.1016/j.cpr.2007.02.005

2. Ferrari AJ, Somerville AJ, Baxter AJ, Norman R, Patten SB, Vos T, et al. Global variation in the prevalence and incidence of major depressive disorder: A systematic review of the epidemiological literature. *Psychol Med*. (2013) 43:471–81. doi: 10.1017/S0033291712001511

3. Chisholm D, Sweeny K, Sheehan P, Rasmussen B, Smit F, Cuijpers P, et al. Scaling-up treatment of depression and anxiety: A global return on investment analysis. *Lancet Psychiatry*. (2016) 3:415–24. doi: 10.1016/S2215-0366(16)30024-4

4. Strekalova T, Liu Y, Kiselev D, Khairuddin S, Chiu JLY, Lam J, et al. Chronic mild stress paradigm as a rat model of depression: Facts, artifacts, and future perspectives. *Psychopharmacology*. (2022) 239:663–93. doi: 10.1007/s00213-021-05982-w

5. Pigott HE, Leventhal AM, Alter GS, Boren JJ. Efficacy and effectiveness of antidepressants: Current status of research. *Psychother Psychosom*. (2010) 79:267–79. doi: 10.1159/000318293

6. Fogaça MV, Duman RS. Cortical GABAergic dysfunction in stress and depression: New insights for therapeutic interventions. *Front Cell Neurosci*. (2019) 13:87. doi: 10.3389/fncel.2019.00087

7. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: Beyond monoamines. *Nat Rev Neurosci*. (2006) 7:137–51. doi: 10.1038/nrn1846

8. Hillhouse TM, Porter JH. A brief history of the development of antidepressant drugs: From monoamines to glutamate. *Exp Clin Psychopharmacol*. (2015) 23:1–21. doi: 10.1037/a0038550

9. Peng GJ, Tian JS, Gao XX, Zhou YZ, Qin XM. Research on the pathological mechanism and drug treatment mechanism of depression. *Curr Neuropharmacol*. (2015) 13:514–23. doi: 10.2174/1570159x1304150831120428

10. Rius-Pérez S, Torres-Cuevas I, Millán I, Ortega ÁL, Pérez S. PGC-1α, inflammation, and oxidative stress: An integrative view in metabolism. *Oxid Med Cell Longev*. (2020) 2020:2020:1452696. doi: 10.1155/2020/1452696

11. Phillips C, Fahimi A. Immune and neuroprotective effects of physical activity on the brain in depression. *Front Neurosci*. (2018) 12:498. doi: 10.3389/fnins.2018.00498

12. Wójtowicz S, Strosznajder AK, Jeżyna M, Strosznajder JB. The novel role of PPAR alpha in the brain: Promising target in therapy of Alzheimer's disease and other neurodegenerative disorders. *Neurochem Res*. (2020) 45:972–88. doi: 10.1007/s11064-020-02993-5

13. Jornayvaz FR, Shulman GI. Regulation of mitochondrial biogenesis. *Essays Biochem*. (2010) 47:69–84. doi: 10.1042/bse0470069

14. Zhao Y, Zhang J, Zheng Y, Zhang Y, Zhang XJ, Wang H, et al. NAD+improves cognitive function and reduces neuroinflammation by ameliorating mitochondrial damage and decreasing ROS production in chronic cerebral hypoperfusion models through Sirt1/PGC-1α pathway. *J Neuroinflammation*. (2021) 18:207. doi: 10.1186/s12974-021-02250-8

15. Johri A. Disentangling mitochondria in Alzheimer's disease. *Int J Mol Sci*. (2021) 22:11520. doi: 10.3390/ijms222111520

16. Zhang H, Dong Z, Lu Y, Yu H, Ma K. Research progress on classical traditional Chinese medicine formula lily bulb and Rehmannia decoction in the treatment of depression. *Biomed Pharmacother*. (2019) 112:108616. doi: 10.1016/j.biopha.2019.108616

17. Wan R, Song R, Fan Y, Li L, Zhang J, Zhang B, et al. Efficacy and safety of Chaihu Jia Longgu Muli decoction in the treatment of poststroke depression: A systematic review and meta-analysis. *Evid Based Complement Alternat Med*. (2021) 2021:7604537–13. doi: 10.1155/2021/7604537

18. Shi M, Xu X, Chen L, Lan G, Jiang W, Wang J, et al. Efficacy of Zishui Qinggan decoction in treatment of perimenopause women with syndrome of renal deficiency and hepatic stagnation and impact on relevant indexes. *Chin J Gen Pract*. (2019) 17:2059–62. doi: 10.16766/j.cnki.issn.1674-4152.001127

19. Liu X, Tian L. Experience in clinical application of Zishui Qinggan drink. *Guiding J Tradit Chin Med Phar*. (2016) 2:108–9. doi: 10.13862/j.cnki.cn43-1446/r.2016.09.039

20. Sun M, Cui S, Zhang C, Sun S. Clinical study of Zishui Qinggan decoction in the treatment of depression. *Acta Chin Med*. (2015) 30:1056–7. doi: 10.16368/j.issn.1674-8999.2015.07.364

21. Hao H, Jiang H, Kang Z. Research of combination of Chinese traditional and western medicinein in treating involutional melancholia. *Acta Chin Med*. (2017) 32:1018–20. doi: 10.16368/j.issn.1674-8999.2017.06.267

22. Vitor-de-Lima SM, Medeiros LB, Benevides RDL, Dos Santos CN, Lima da Silva NO, Guedes RCA. Monosodium glutamate and treadmill exercise: Anxiety-like behavior and spreading depression features in young adult rats. *Nutr Neurosci*. (2019) 22:435–43. doi: 10.1080/1028415X.2017.1398301

23. Yang F, Zhu W, Cai X, Zhang W, Yu Z, Li X, et al. Minocycline alleviates NLRP3 inflammasome-dependent pyroptosis in monosodium glutamate-induced depressive rats. *Biochem Biophys Res Commun*. (2020) 526:553–9. doi: 10.1016/j.bbrc.2020.02.149

24. Palikaras K, Tavernarakis N. Regulation and roles of mitophagy at synapses. *Mech Ageing Dev*. (2020) 187:111216. doi: 10.1016/j.mad.2020.111216

25. Licznerski P, Duman RS. Remodeling of axo-spinous synapses in the pathophysiology and treatment of depression. *Neuroscience*. (2013) 251:33–50. doi: 10.1016/j.neuroscience.2012.09.057

26. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: Potential therapeutic targets. *Science*. (2012) 338:68–72. doi: 10.1126/science.1222939

27. Chidambaram SB, Rathipriya AG, Bolla SR, Bhat A, Ray B, Mahalakshmi AM, et al. Dendritic spines: Revisiting the physiological role. *Prog Neuro-Psychopharmacol Biol Psychiatry*. (2019) 92:161–93. doi: 10.1016/j.pnpbp.2019.01.005

28. Moench KM, Maroun M, Kavushansky A, Wellman C. Alterations in neuronal morphology in infralimbic cortex predict resistance to fear extinction following acute stress. *Neurobiol Stress*. (2015) 3:23–33. doi: 10.1016/j.ynstr.2015.12.002

29. Martin KP, Wellman CL. NMDA receptor blockade alters stress-induced dendritic remodeling in medial prefrontal cortex. *Cereb Cortex*. (2011) 21:2366–73. doi: 10.1093/cercor/bhr021

30. Bollinger JL, Bergeon Burns CM, Wellman CL. Differential effects of stress on microglial cell activation in male and female medial prefrontal cortex. *Brain Behav Immun*. (2016) 52:88–97. doi: 10.1016/j.bbi.2015.10.003

31. Song Z, Bian Z, Zhang Z, Wang X, Zhu A, Zhu G. Astrocytic Kir 4.1 regulates NMDAR/calpain signaling axis in lipopolysaccharide-induced depression-like behaviors in mice. *Toxicol Appl Pharmacol*. (2021) 429:115711. doi: 10.1016/j.taap.2021.115711

32. Cui Y, Cao K, Lin H, Cui S, Shen C, Wen W, et al. Early-life stress induces depression-like behavior and synaptic-plasticity changes in a maternal separation rat model: Gender difference and metabolomics study. *Front Pharmacol*. (2020) 11:102. doi: 10.3389/fphar.2020.00102

33. Kramer P, Bressan P. Our (Mother's) mitochondria and our mind. *Perspect Psychol Sci*. (2018) 13:88–100. doi: 10.1177/1745691617718356

34. Kim Y, Vadodaria KC, Lenkei Z, Kato T, Gage FH, Marchetto MC, et al. Mitochondria, metabolism, and redox mechanisms in psychiatric disorders. *Antioxid Redox Signal*. (2019) 31:275–317. doi: 10.1089/ars.2018.7606

35. Picard M, McEwen BS, Epel ES, Sandi C. An energetic view of stress: Focus on mitochondria. *Front Neuroendocrinol*. (2018) 49:72–85. doi: 10.1016/j.yfrne.2018.01.001

36. Allen J, Romay-Tallon R, Brymer KJ, Caruncho HJ, Kalynchuk LE. Mitochondria and mood: Mitochondrial dysfunction as a key player in the manifestation of depression. *Front Neurosci*. (2018) 12:386. doi: 10.3389/fnins.2018.00386

37. Rappeneau V, Wilmes L, Touma C. Molecular correlates of mitochondrial dysfunctions in major depression: Evidence from clinical and rodent studies. *Mol Cell Neurosci*. (2020) 109:103555. doi: 10.1016/j.mcn.2020.103555

38. Bauer ME, Teixeira AL. Inflammation in psychiatric disorders: What comes first? *Ann N Y Acad Sci*. (2019) 1437:57–67. doi: 10.1111/nyas.13712

39. De Gaetano A, Solodka K, Zanini G, Selleri V, Mattioli AV, Nasi M, et al. Molecular mechanisms of mtDNA-mediated inflammation. *Cells*. (2021) 10:2898. doi: 10.3390/cells10112898

40. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav Immun*. (2011) 25:181–213. doi: 10.1016/j.bbi.2010.10.015

41. Beurel E, Toups M, Nemeroff CB. The bidirectional relationship of depression and inflammation: Double trouble. *Neuron*. (2020) 107:234–56. doi: 10.1016/j.neuron.2020.06.002

42. Rohleder N. Stress and inflammation – The need to address the gap in the transition between acute and chronic stress effects. *Psychoneuroendocrinology*. (2019) 105:164–71. doi: 10.1016/j.psyneuen

43. Andrieux P, Chevillard C, Cunha-Neto E, Nunes JPS. Mitochondria as a cellular hub in infection and inflammation. *Int J Mol Sci*. (2021) 22:11338. doi: 10.3390/ijms222111338

44. Martínez-Redondo V, Pettersson AT, Ruas JL. The hitchhiker's guide to PGC-1α isoform structure and biological functions. *Diabetologia*. (2015) 58:1969–77. doi: 10.1007/s00125-015-3671-z

45. Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: A potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci U S A*. (1994) 91:1309–13. doi: 10.1073/pnas.91.4.1309

46. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies. *Biochim Biophys Acta Mol basis Dis*. (2017) 1863:1066–77. doi: 10.1016/j.bbadis.2016.11.010

47. Kuczynska Z, Metin E, Liput M, Buzanska L. Covering the role of PGC-1α in the nervous system. *Cells*. (2021) 11:111. doi: 10.3390/cells11010111

48. Cheng A, Wan R, Yang JL, Kamimura N, Son TG, Ouyang X, et al. Involvement of PGC-1α in the formation and maintenance of neuronal dendritic spines. *Nat Commun*. (2012) 3:1250. doi: 10.1038/ncomms2238

49. Zhou H, Gan M, Jin X, Dai M, Wang Y, Lei Y, et al. miR-382 inhibits breast cancer progression and metastasis by affecting the M2 polarization of tumor-associated macrophages by targeting PGC-1α. *Int J Oncol*. (2022) 61:126. doi: 10.3892/ijo.2022.5416

50. Yang X, Xu S, Qian Y, Xiao Q. Resveratrol regulates microglia M1/M2 polarization via PGC-1α in conditions of neuroinflammatory injury. *Brain Behav Immun*. (2017) 64:162–72. doi: 10.1016/j.bbi.2017.03.003

51. Fu X, Jiao J, Qin T, Yu J, Fu Q, Deng X, et al. A new perspective on ameliorating depression-like behaviors: Suppressing neuroinflammation by upregulating PGC-1α. *Neurotox Res*. (2021) 39:872–85. doi: 10.1007/s12640-020-00292-z

52. Wu Y, Sun F, Guo Y, Zhang Y, Li L, Dang R, et al. Curcumin relieves chronic unpredictable mild stress-induced depression-like behavior through the PGC-1α/FNDC5/BDNF pathway. *Behav Neurol*. (2021) 2021:2630445–13. doi: 10.1155/2021/2630445

53. Wang YS, Shen CY, Jiang JG. Antidepressant active ingredients from herbs and nutraceuticals used in TCM: Pharmacological mechanisms and prospects for drug discovery. *Pharmacol Res*. (2019) 150:104520. doi: 10.1016/j.phrs.2019.104520

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 25 May 2023
DOI 10.3389/fneur.2023.1083066

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Mengxing Wang,
Shanghai University of Medicine and Health Sciences, China
Ruiwang Huang,
South China Normal University, China

*CORRESPONDENCE
Fangyuan Cui
✉ fangyuancui2011@hotmail.com

RECEIVED 28 October 2022
ACCEPTED 09 May 2023
PUBLISHED 25 May 2023

CITATION
Lin D, Gao J, Lu M, Han X, Tan Z, Zou Y and Cui F (2023) Scalp acupuncture regulates functional connectivity of cerebral hemispheres in patients with hemiplegia after stroke. *Front. Neurol.* 14:1083066. doi: 10.3389/fneur.2023.1083066

Scalp acupuncture regulates functional connectivity of cerebral hemispheres in patients with hemiplegia after stroke

Dan Lin[1], Jinyang Gao[1], Mengxin Lu[1], Xiao Han[1], Zhongjian Tan[2], Yihuai Zou[1] and Fangyuan Cui[1]*

[1]Department of Neurology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China, [2]Department of Radiology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China

Background: Stroke is a common cause of acquired disability on a global scale. Patients with motor dysfunction after a stroke have a reduced quality of life and suffer from an economic burden. Scalp acupuncture has been proven to be an effective treatment for motor recovery after a stroke. However, the neural mechanism of scalp acupuncture for motor function recovery remains to be researched. This study aimed to investigate functional connectivity (FC) changes in region of interest (ROI) and other brain regions to interpret the neural mechanism of scalp acupuncture.

Methods: Twenty-one patients were included and randomly divided into patient control (PCs) and scalp acupuncture (SAs) groups with left hemiplegia due to ischemic stroke, and we also selected 20 matched healthy controls (HCs). The PCs were treated with conventional Western medicine, while the SAs were treated with scalp acupuncture (acupuncture at the right anterior oblique line of vertex temporal). All subjects received whole-brain resting-state functional magnetic resonance imaging (rs-fMRI) scan before treatment, and the patients received a second scan after 14 days of treatment. We use the National Institutes of Health Stroke Scale (NIHSS) scores and the analyses of resting-state functional connectivity (RSFC) as the observational indicators.

Results: The contralateral and ipsilateral cortex of hemiplegic patients with cerebral infarction were associated with an abnormal increase and decrease in basal internode function. An abnormal increase in functional connectivity mainly exists in the ipsilateral hemisphere between the cortex and basal ganglia and reduces the abnormal functional connectivity in the cortex and contralateral basal ganglia. Increased RSFC was observed in the bilateral BA6 area and bilateral basal ganglia and the connectivity between bilateral basal ganglia nuclei improved. However, the RSFC of the conventional treatment group only improved in the unilateral basal ganglia and contralateral BA6 area. The RSFC in the left middle frontal gyrus, superior temporal gyrus, precuneus, and other healthy brain regions were enhanced in SAs after treatment.

Conclusion: The changes in functional connectivity between the cerebral cortex and basal ganglia in patients with cerebral infarction showed a weakening of the bilateral hemispheres and the enhancement of the connections between the hemispheres. Scalp acupuncture has the function of bidirectional regulation, which makes the unbalanced abnormal brain function state restore balance.

KEYWORDS

stroke, resting-state functional magnetic resonance imaging, hemiplegia, scalp acupuncture, functional connectivity

Background

With the improving medical conditions, stroke remains a leading cause of death and long-term disability worldwide. Epidemiologic evidence shows that the average global lifetime risk of stroke has increased from 22.8 to 24.9% over the past 20 years; in the United States, the prevalence of stroke in adults is 3.0% (median). On average, someone suffers a stroke nearly every 40 s, and rates increase with age in both men and women (1). In China, stroke has become the leading cause of death in 2019 (2, 3). Hemiplegia is one of the most common sequelae after stroke, which directly affects the quality of work and life of patients and imposes a heavy economic burden on society and families (4).

Motor impairment is the most common symptom of stroke. In recent years, motor impairment is found to be relevant to abnormal functional connectivity in several functional magnetic resonance imaging (fMRI) studies (5–7). The correlations of the interhemispheric FC changes during motor function injury and rehabilitation have been observed widely, especially between the brain regions directly related to the motor network. Many previous studies showed (8, 9) that interhemispheric functional connectivity in the motor network is a potential neurobiological marker for recovery after stroke rehabilitation. However, the recovery of motor function and the reconstruction of the movement patterns are also relative to a wider range of functions, such as perception, memory, learning, executive functioning, and emotion management (10–12).

Spontaneous behavioral recovery usually occurs within weeks to months after stroke, which is often incomplete and affected by age, previous comorbidities, and the size and location of the injury (13). A large number of studies have demonstrated that appropriate rehabilitation therapy could promote the recovery of motor function, thus enhancing the functional recovery of damaged areas by promoting neuroplastic changes. The mechanisms of these therapies are similar to the mechanisms observed in spontaneous recovery (14). Some researches show that acupuncture may be possible to promote motor recovery by coordinating functional activities of more extensive brain regions, which initially were involved in executive function processes such as planning, initiation, and attention (15, 16). At present, scalp acupuncture is widely used in the clinical treatment of stroke and rehabilitation of stroke (17). According to the traditional Chinese medical theory, the head is where the IQ and the meridian merge. Scalp acupuncture can improve the excitability of the central nervous system, effectively improve cerebral hemodynamics and brain tissue metabolism, and then promote the recovery of motor function of patients. In this study, we evaluated the role of Scalp acupuncture treatment in patients with hemiplegia after stroke and analyzed the changes in RSFC. This study may help to evaluate the mechanism of scalp acupuncture treatment in patients with hemiplegia.

Materials and methods

Participants

This study was approved by Dongzhimen Hospital Affiliated with the Beijing University of Chinese Medicine Institutional Review Boards (approval number: ECSL-BDY-2014-16).

Twenty-one patients with left hemiplegia due to ischemic stroke were recruited from the Dongzhimen Hospital (Beijing, China) between February 2014 and December 2017. In addition, 20 healthy subjects were recruited as a control group with matched age, sex, and education levels. Patients were randomly classified by using the random number table method.

Inclusion criteria: (a) patients had a clinically diagnosed by computed tomography or MRI first episode of ischemic stroke involving unilateral partial anterior circulation infarction; (b) patients were between 40 and 75 years old and right-handed; (c) patients' course was between 2 weeks and 6 months after the onset of stroke; (d) patients had the stable physical condition without disorders in consciousness or speech and can understand consenting and study-related instructions; (e) patients had signed the informed consent form. Exclusion criteria: (a) patients with a course of disease longer than 6 months or in the acute phase of disease progression; (b) patients with sensory aphasia, severe dementia, psychosis, and so on, which may affect the experiment; (c) patients with a cardiac pacemaker, stent, bypass surgery, or otherwise, which were not available for magnetic resonance examination.

Interventional method

Both groups were given conventional Western medicine treatment, such as blood pressure-lowering treatment, lipid-lowering and stabilizing agents, and anti-platelet.

Acupuncture was applied at the anterior oblique line of vertex temporal (MS6) according to the International Standardization Scheme for Scalp Acupuncture Points (18). First, we sterilize the skin of patients and our hands, and then, the stainless steel needles (0.25×40 mm) were inserted at angles of almost 15° with respect to the epicranial aponeurosis. When the needle goes into the scalp to a certain depth, the needles were twisted at a frequency of 200 turns per min, causing a deqi sensation. Twist the needles every 10 min for 30 min. Once a day for 5 consecutive days as a course of treatment.

Study procedure

All subjects underwent the National Institute of Health stroke scale (NIHSS) evaluations and their first rs-fMRI examination at baseline; patients with hemiplegia underwent the second NIHSS evaluation and rs-fMRI examination after scalp acupuncture treatment. The flow chart of this study is shown in Figure 1.

Image data acquisition

MRI data were acquired using a 3.0 Tesla Siemens scanner (MAGNETOM Verio Siemens Medical Systems, Erlangen, Germany) with a 32-channel head coil at the Dongzhimen Hospital, Beijing, China. For rs-fMRI scans, participants were instructed to keep their eyes closed and stay awake without performing any cognitive tasks. The imaging parameters of the EPI sequence were as follows: repetition time (TR)

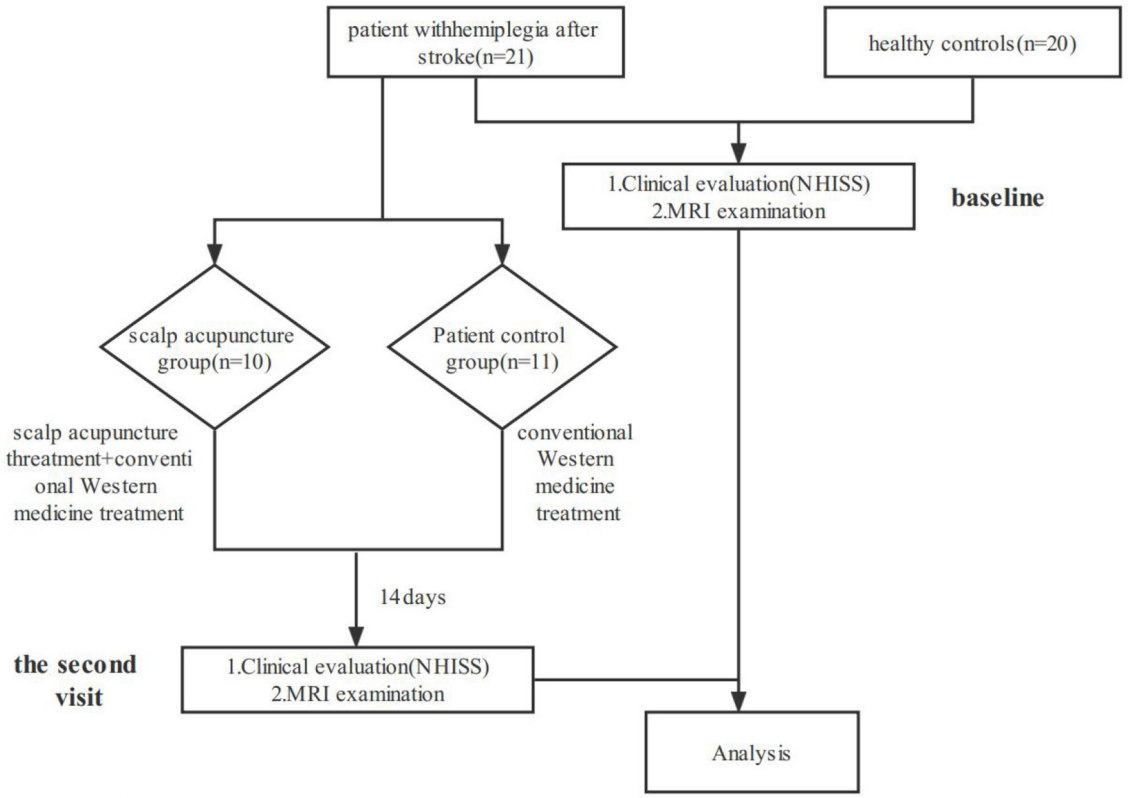


FIGURE 1
Flow chart of the current study.

= 2,000 ms, field of view (FOV) = 250 × 250 mm², echo time (TE) = 30ms, slice number = 31, thickness = 3.5 mm, flip angle = 90°, and matrix size = 64 × 64. The single-acquisition time was 620 s. High-resolution structural images were acquired through a magnetization-prepared rapid acquisition with gradient-echo (MPRAGE) sequence with the following parameters: TR/TE = 2,700/2.97 ms, FOV = 250 × 250 mm², matrix size = 256 × 256, flip angle = 7°, slice number = 176, and slice thickness = 1 mm. The single-acquisition time was 377 s.

FMRI data analysis

The preprocessing of fMRI data was performed with SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB 8.2 (https://ww2.mathworks.cn/products/matlab.html). First, we preprocessed the FMRI data, mainly including the following steps: (a) the data of the first 10 time points were deleted to ensure the stability of MRI data; (b) slice time correction and motion correction (over 1 mm or 1° were excluded); (c) spatial normalization; (d) smoothing of the data with an 8-mm full width at half maximum (FWHM) kernel; (e) detrend and filtering, band-pass filtering was performed with a frequency window of 0.008–0.09 Hz; and (f) in addition to signals of interest, confounding factors were eliminated by segmentation of white matter and cerebrospinal fluid (CSF) regions.

Seven regions of the subcortex that were widely used in previous studies were examined (19–22). These seven seeds, including the caudate nucleus, putamen, nucleus accumbens, globus pallidus, subthalamic nucleus, substantia nigra, and thalamus, were extracted using WFU-Pick Atlas software (https://www.nitrc.org/projects/wfu_pickatlas).

Functional connectivity analysis was carried out using a seed-based approach in the CONN16b (https://www.nitrc.org/projects/conn/). The first-order correlation map was generated by extracting the remaining BOLD time process from each striatum seed region and calculating its Pearson correlation coefficient with the whole brain voxel time process. The correlation coefficients were converted to "Z" values by Fisher conversion, and their normality was improved to enable them to be used in improved second-order general linear model analysis. In addition, the differences in FC between ROI points and the whole brain between patients and HCs were compared by the two-samples t-test in the secondary group analysis. Voxel threshold $p < 0.005$ (uncorrected) and cluster level $p < 0.05$ (FDR corrected) were considered as statistical differences. SPSS18.0 statistical software package was used for clinical data analysis; the chi-square test was used for counting data, t-test was used for measurement data consistent with normal distribution

TABLE 1 Demographics and clinical characteristics of patients with hemiplegia after stroke and healthy controls (HCs).

	HCs ($n = 20$)	SAs ($n = 11$)	PCs ($n = 10$)
Age	57.4 ± 8.39	68.4 ± 4.5	57.1 ± 9.8
Sex (female/male)	7/13	2/8	3/8
NIHSS score	NA	5.0 ± 2.75	5.9 ± 2.62

NA, not available. The data were expressed as (Mean ± SD).

and Mann–Whitney test was used for non-parametric test if not consistent with normal distribution. $P < 0.05$ was considered statistically significant.

Results

Demographic and clinical data

The demographic and clinical characteristics of the participants are summarized in Table 1. There were no significant differences between the patients and HCs in terms of age ($p = 0.079$) and gender ($p = 0.444$), and between the scalp acupuncture (SAs) group and patient control (PCs) group in terms of gender ($p = 0.696$). There were significant differences between SAs and PCs in terms of age ($p = 0.003$). At the same time, no significant differences ($p = 0.410$) were found between the SAs and PCs in the NIHSS scores before the first scan, while significant differences were found ($p = 0.027$) after 14-day treatment (Table 1).

Functional connectivity results

Decrease RSFC between contralateral hemispheres

Caudate nucleus

Significantly decreased RSFC was found between the left caudate and right posterior cerebellar lobe, right caudate nucleus, right temporal lobe, right medial frontal gyrus, left parietal lobe, left superior frontal gyrus, and left temporal lobe in stroke patients compared to the HCs. Decreased RSFC was observed between the right caudate and left middle frontal gyrus, left thalamus, left frontal lobe, left posterior cerebellum, left cingulate gyrus, and right middle frontal gyrus (Figures 2A, B).

Putamen

Compared to HCs, decreased RSFC was also observed between the putamen and contralateral areas in stroke patients with left hemiplegia. Interestingly, both sides showed weakened RSFC between the putamen and contralateral thalamus and cingulate gyrus. In other words, there was a significantly reduced RSFC of the left putamen with the right thalamus and right cingulate gyrus and of the right putamen with the left thalamus and left cingulate gyrus in patients with left hemiparesis (Figures 2C, D).

Thalamus

Decreased RSFC of the left thalamus and the right frontal lobe, right temporal lobe, and right cingulate gyrus were observed in patients with stroke. Furthermore, there was decreased RSFC between the right thalamus and the left parahippocampal gyrus, left cingulate gyrus, right parietal lobe, and right anterior cuneal lobe (Figures 2E, F).

Increased RSFC in the ipsilateral hemisphere

Contrary to the weakened RSFC, significantly increased RSFC was found in the patients located within the same side of the seed points compared with HCs. There was increased RSFC between the right putamen and the right parietal lobe; increased RSFC between the right nucleus accumbens and the right pallidum; increased RSFC between the left globus pallidus and the left lateral globus pallidus and the middle occipital gyrus; and increased RSFC of the right globus pallidus with the right putamen and right parietal lobe (Figure 3).

Changes of RSFC in patients after treatment

Patients treated with acupuncture

Having received a 14-day treatment with scalp acupuncture, patients in the acupuncture group showed both increased and decreased RSFC of different seeds.

There were increased RSFC between the left caudate nucleus and the left median frontal gyrus, increased RSFC between the right globus pallidus and the left frontal gyrus, decreased RSFC between the right thalamus and the left temporal lobe, and decreased RSFC between the left caudate nucleus and the left superior temporal gyrus and left marginal lobe (Table 2).

Patients without acupuncture

Compared with HCs, increased RSFC of the right globus pallidus with the right suboccipital gyrus, the left superior frontal gyrus, and decreased RSFC of the left globus pallidus with the right superior frontal gyrus were observed in patients after 14-day non-acupuncture treatment (Table 3).

RSFC between SAs and PCs

Compared with the PCs, there were significant differences in extensive brain regions of RSFC in SAs after treatment, and their localizations were as follows: (a) increased RSFC of the right putamen with the right posterior central gyrus and insula; (b) increased RSFC of the left thalamus with the right thalamus, the occipital thalamus, and the precentral gyrus; (c) decreased RSFC of the left globus pallidus with the left superior temporal gyrus; (d) decreased RSFC of the left caudate nucleus with the right lingual gyrus; (e) decreased RSFC of the left thalamus with the left middle frontal gyrus and precuneus (Figure 4).

Discussion

In this study, we found that there were significant differences between SAs and PCs in the NIHSS scores after scalp acupuncture

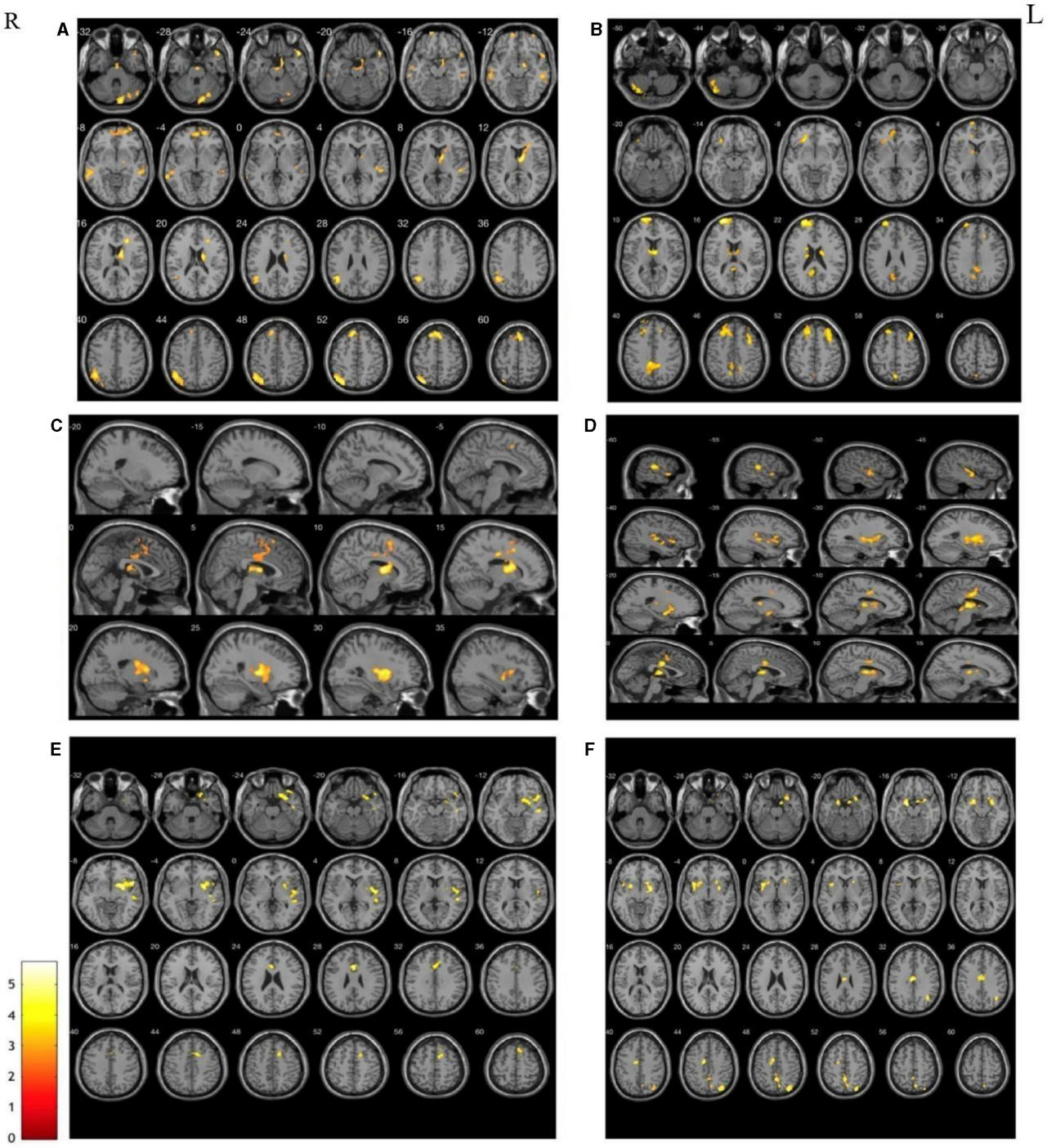


FIGURE 2
The areas with the significantly reduced between HCs and patients in terms of the changes in functional connectivity before treatment. Decreased RSFC were found in both left and right seeds, including caudate nucleus **(A, B)**, putamen **(C, D)** and thalamus **(E, F)**. Interestingly, the weakened connectivity areas mainly located on the contralateral cerebral hemisphere of the seed point.

treatment. This suggested that scalp acupuncture may have a great effect on the improvement of neurological dysfunction in patients with hemiplegia, which is consistent with the results of previous studies (23). At the same time, this study provides new insights into the functional connectivity of the cerebral hemispheres after a stroke and advances our understanding of the functional reorganization of the brain after a stroke and the promotion of motor rehabilitation by scalp acupuncture. In addition to local tissue damage, changes in functional connectivity between intact structural regions that connect the site of injury

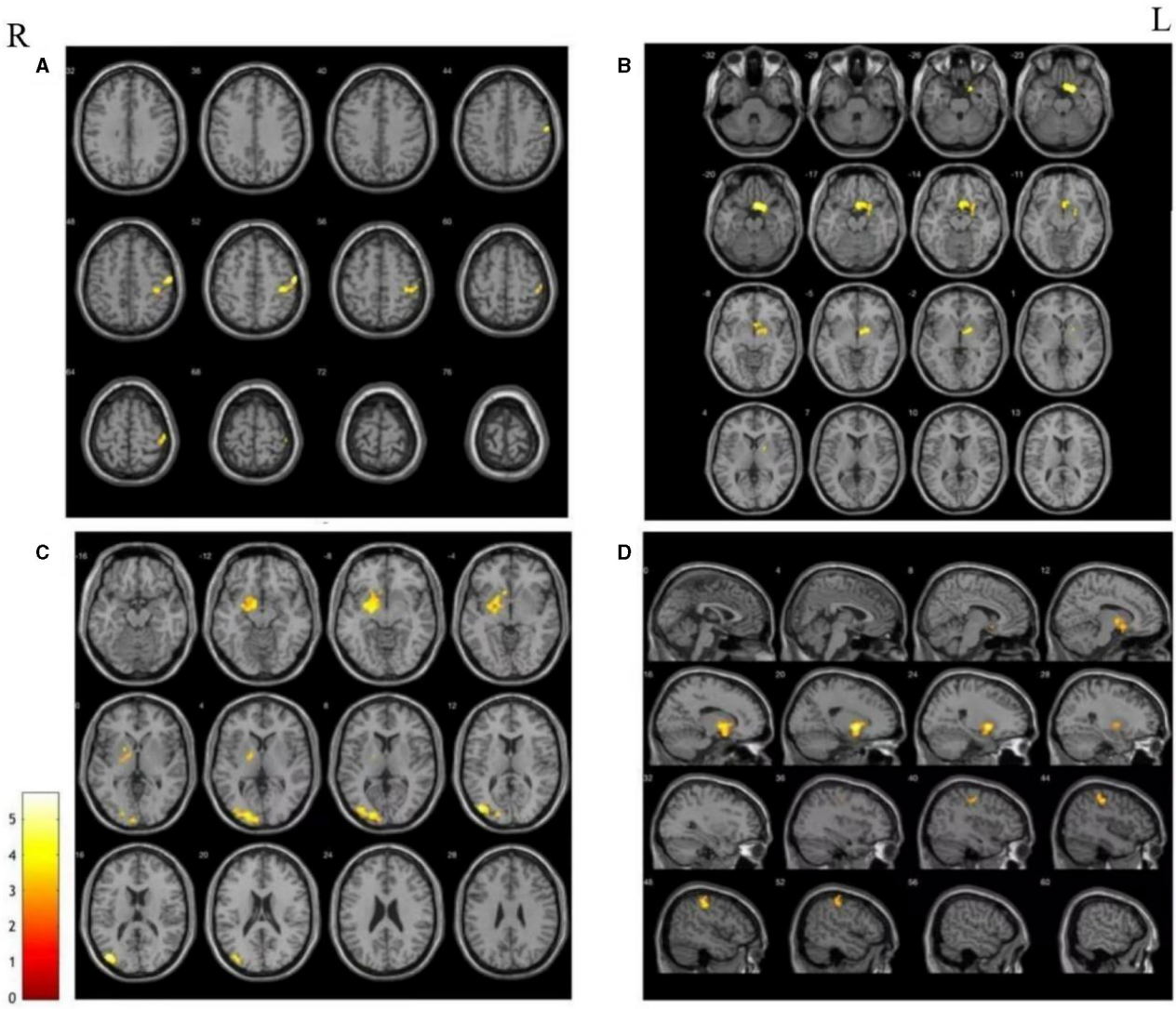


FIGURE 3
The areas with the significantly increase between HCs and patients in terms of the changes in functional connectivity before treatment. **(A)** Increased RSFC between right putamen and right parietal; **(B)** Increased RSFC between right nucleus accumbens and the right pallidum; **(C)** increased RSFC between the left globus pallidus and the left lateral globus pallidus and the middle occipital gyrus; **(D)** increased RSFC of the right globus pallidus with the right putamen and right parietal lobe.

could also cause motor dysfunction in patients with spotty brain injuries (24).

The functional connectivity strength of each brain voxel is closely related to behavioral deficits and is an important predictor of post-stroke recovery. Compared with the HCs, the FC between basal ganglia nuclei with the contralateral brain regions was significantly decreased in patients with hemiplegia after stroke, especially in similar areas of the two brain hemispheres, which is consistent with previous research (25, 26). Damage of the unilateral brain in patients can affect the exchanges and cooperation between the hemispheres of the brain. At the same time, we also found that the FC was increased between the sensory and visual-related brain areas in the ipsilateral hemisphere on both affected and healthy sides in hemiplegia patients, which suggests that the ability of patients to receive and process the sensory information of the hemiplegic side and observe the external environment was enhanced after cerebral infarction. In this study, patients were all in the convalescence period, which had certain functional recovery and compensation. The enhanced FC in the ipsilateral hemisphere of the brain was also consistent with the brain functional state of functional injury and repair in the period of recovery. In summary, the regularity changes of FC in the brain revealed the abnormal motor network after the stroke and also reflected the functional reorganization during recovery.

In the present study, the RSFC in the contralateral auxiliary motor area was enhanced in both the lesion and the healthy side of the basal ganglia after scalp acupuncture treatment. However, the

TABLE 2 Brain regions with increased and reduced functional connectivity after treatment in the SAs.

Seed		Region	Hem/BA	*T*	*Z*	Voxels	Coordinate MNI (mm)		
							X	*Y*	*Z*
Increase	Cau(L)	Middle frontal gyrus	R/BA9,6	4.34	3.59	566	50	24	34
	Glo(R)	Superior frontal gyrus	L/BA6,10	4.99	3.90	375	−26	27	−2
Reduce	Tha(L)	Temporal lobe	L/BA39	6.12	4.44	510	−44	−58	22
	Cau(L)	Superior temporal gyrus	L/BA39,40	5.95	4.37	489	−42	−58	28
		Limbic lobe	L/BA31	4.47	3.62	379	−4	−52	32

R, right; L, left; BA, Brodmann area; Cau, caudate nucleus; Tha, thalamus; Glo, globus pallidus.

TABLE 3 Brain regions with increased and reduced functional connectivity after treatment in the PCs.

Seed		Region	Hem/BA	*T*	*Z*	Voxels	Coordinate MNI (mm)		
							x	*y*	*z*
Increase	Glo(R)	Inferior occipital gyrus	R/BA17,18	5.96	4.37	482	30	−90	−18
		Superior frontal gyrus	L/BA6	4.66	3.73	420	−22	58	−4
Reduce	Glo(L)	Superior frontal gyrus	R/BA6	4.88	3.85	343	18	−8	72

R, right; L, left; BA, Brodmann area; Glo, globus pallidus.

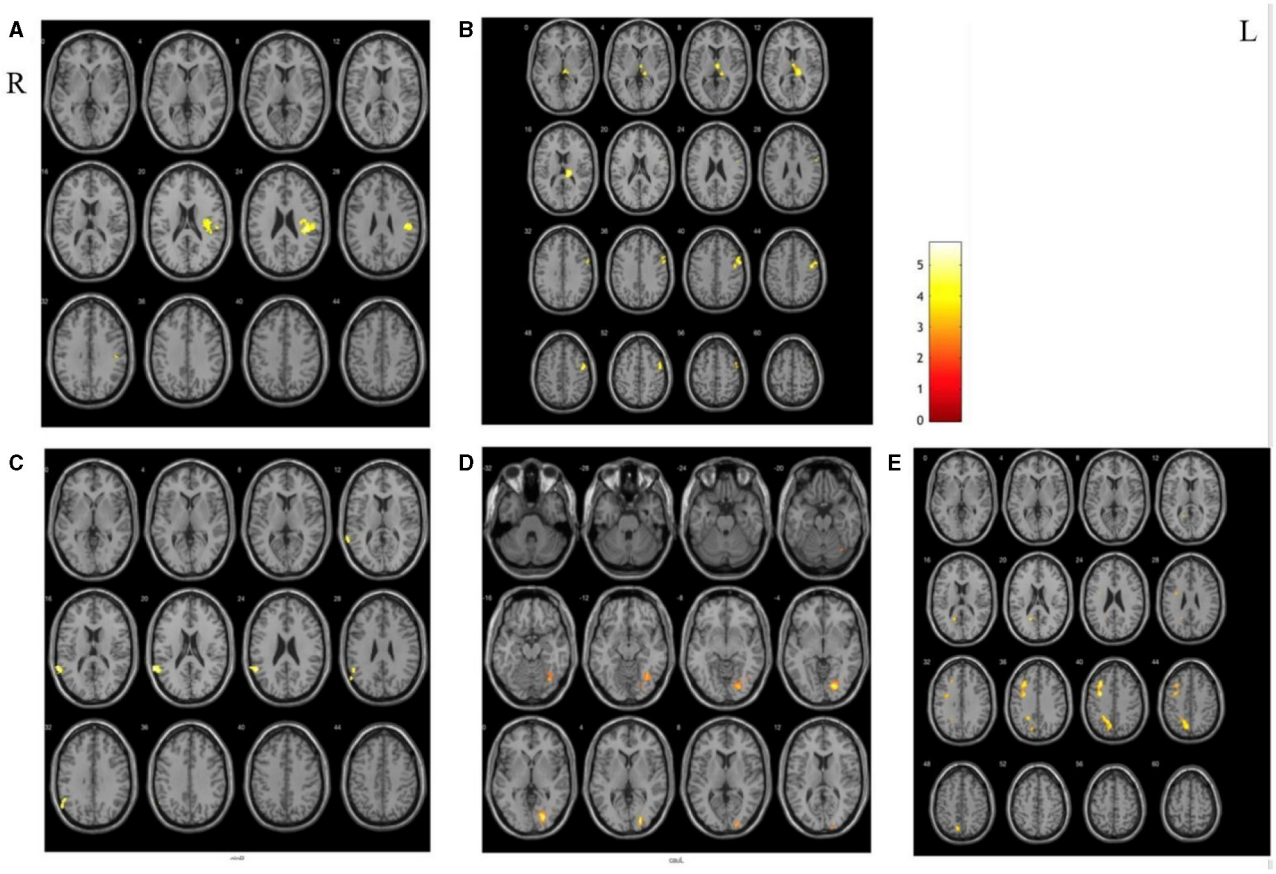


FIGURE 4
The different areas of functional connectivity between SAs and PCs after treatment. **(A, B)** Increased RSFC between SAs and PCs; **(C–E)** deceased RSFC between SAs and PCs.

functional connectivity between the basal ganglia of the lesion and the contralateral BA6 was enhanced after conventional treatment in the patient control group. The diversity of functions involving BA6, probably the largest Brodmann's area, is not surprising. The BA6 area, including the premotor cortex and supplementary motor area, affects the realization of motor function through the regulation of the primary motor cortex and is also involved in language, memory, attention, and executive functions (27, 28). Both animal experiments and clinical trials suggest that activation of the BA6 region contributes to functional reorganization after stroke (29, 30). Another clinical trial of scalp acupuncture for acute ischemic stroke suggested that after scalp acupuncture, the voxel-mirrored homotopic connectivity value of bilateral BA6 and BA8 increased significantly, and the synchronization of functional activities between bilateral BA6 and BA8 was enhanced (15). A study of regional homogeneity between acupuncture and ischemic stroke found that both groups showed higher ReHo in BA6 (31). The above studies were similar to the results of this study, which showed that scalp acupuncture could significantly improve the FC of the cortical–subcortical motor pathway and promote the recovery of motor function in patients with cerebral infarction.

The significance of hyperexcitability ipsilateral motor responses from the unaffected hemisphere for motor recovery has been considered to be doubtful (32). Although the enhanced activation of the contralateral brain region after stroke can promote functional rehabilitation in the short term, it could also cause wrong movement patterns such as associated movement, which has adverse effects on the rehabilitation of the neurological function of the damaged hemisphere (33). The better the prognosis for rehabilitation, the more the degree of abnormal hyperactivation of the healthy hemisphere is reduced during rehabilitation and the more normalized the imbalance in bilateral brain function due to lesion injury (26, 34). In this study, some brain regions in the unaffected hemisphere showed enhanced functional connectivity, while the functional connectivity between the left basal ganglia, left temporal lobe, and left marginal lobe was weakened after scalp acupuncture treatment. In addition, compared with the patient control group, the scalp acupuncture group showed a greater degree of functional connectivity decline in the left middle frontal gyrus, superior temporal gyrus, precuneus, and other healthy brain regions. The above results suggest that scalp acupuncture is more beneficial to reduce the compensation of contralateral function. Compared with conventional treatment, scalp acupuncture can significantly reduce the patient's dependence on the compensation of contralateral motor function after stroke and is conducive to the recovery of normal motor patterns of stroke patients. Furthermore, scalp acupuncture treatment not only has a stronger promotion effect on BA6 but also significantly improves the functional connection between the non-motor related brain regions such as the postcentral gyrus and insula. As an important part of the sensorimotor network, the primary somatosensory cortex is located in the postcentral gyrus and is responsible for sensory input (35). The insula involves a wide diversity of functions observed, such as pain, temperature, touch, olfaction, taste, language, memory, and emotion (36). The rehabilitation effect of scalp acupuncture on cortical function is not only limited to the motor area, but also has a positive effect on sensory and cognitive-related functions, and improves nerve function as a whole by promoting the mutual coordination of each brain functional area.

Limitations

There are some limitations in this study. One of the limitations is that there were fewer subjects, and the other is that the patients in this study were in the recovery period of cerebral infarction, but there may be some differences in the remodeling of neural function in different stages of the recovery period. The mechanism of change of head acupuncture treatment may also change differently. Further studies with larger sample sizes and more optimized research designs are needed to confirm our findings.

Conclusion

Scalp acupuncture treatment has a bidirectional adjustment function and promotes rehabilitation by strengthening functional connections of the bilateral motor cortex and weakening abnormal compensatory connections.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine Institutional Review Boards (approval number: ECSL-BDY-2014-16). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DL: writing—original draft preparation, validation, project administration, and supervision. JG: software, investigation, methodology, visualization, and formal analysis. ML: investigation, methodology, and writing—review and editing. XH: data curation and formal analysis. ZT: visualization, supervision, and validation. YZ: methodology, resources, writing—review and editing, and visualization. FC: conceptualization, methodology, writing—review and editing, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Natural Science Foundation of China (grant number 82004303) and the Foundation of Beijing Higher Education (grant number YETP0822).

Acknowledgments

The authors acknowledge Dr. Jing Bai, Dr. Huawei Shi, Dr. Xinglu Dong, Dr. Lifang Ma, Dr. Li Zhou, Dr. Genming Zhang, Dr. Bin MA, and other medical staff of Dongzhimen Hospital for their support and help during this research work. We would like to thank the Magnetic Resonance Department of Dongzhimen Hospital for its facilities and technical support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. *Circulation*. (2020) 141:e139e596. doi: 10.1161/CIR.0000000000000746

2. Ma Q, Li R, Wang L, Yin P, Wang Y, Yan C, et al. Temporal trend and attributable risk factors of stroke burden in China, 1990-2019: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health*. (2021) 6:e897–906. doi: 10.1016/S2468-2667(21)00228-0

3. Ma L, Wang Z, Fan J, Hu S. The Writing Committee of the Report on Cardiovascular Health Diseases in China. *Chin Gen Pract*. (2022) 25:3331–46.

4. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9

5. Calautti C, Naccarato M, Jones PS, Sharma N, Day DD, Carpenter AT, et al. The relationship between motor deficit and hemisphere activation balance after stroke: a 3T fMRI study. *Neuroimage*. (2007) 34:322–31. doi: 10.1016/j.neuroimage.2006.08.026

6. Grefkes C, Fink GR. Connectivity-based approaches in stroke and recovery of function. *Lancet Neurol*. (2014) 13:206–16. doi: 10.1016/S1474-4422(13)70264-3

7. Du J, Hu J, Hu J, Xu Q, Zhang Q, Liu L, et al. Aberrances of cortex excitability and connectivity underlying motor deficit in acute stroke. *Neural Plast*. (2018) 2018:1318093. doi: 10.1155/2018/1318093

8. Hu J, Du J, Xu Q, Yang F, Zeng F, Dai XJ, et al. Altered coupling between motion-related activation and resting-state brain activity in the ipsilesional sensorimotor cortex after cerebral stroke. *Front Neurol*. (2017) 8:339. doi: 10.3389/fneur.2017.00339

9. Lee J, Park E, Lee A, Chang WH, Kim DS, Kim YH. Alteration and role of interhemispheric and intrahemispheric connectivity in motor network after stroke. *Brain Topogr*. (2018) 31:708–19. doi: 10.1007/s10548-018-0644-9

10. Cheng L, Wu Z, Fu Y, Miao F, Sun J, Tong S. Reorganization of functional brain networks during the recovery of stroke: a functional MRI study. *Annu Int*. (2012) 2012:4132–5.

11. Bajaj S, Butler AJ, Drake D, Dhamala M. Functional organization and restoration of the brain motor-execution network after stroke and rehabilitation. *Front Hum Neurosci*. (2015) 9:173. doi: 10.3389/fnhum.2015.00173

12. Guggisberg AG, Koch PJ, Hummel FC, Buetefisch CM. Brain networks and their relevance for stroke rehabilitation. *Clin Neurophysiol*. (2019) 130:1098–124. doi: 10.1016/j.clinph.2019.04.004

13. Cramer SC. Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. *Ann Neurol*. (2008) 63:272–87. doi: 10.1002/ana.21393

14. Cassidy JM, Cramer SC. Spontaneous and therapeutic-induced mechanisms of functional recovery after stroke. *Transl Stroke Res*. (2017) 8:33–46. doi: 10.1007/s12975-016-0467-5

15. Liu H, Jiang Y, Wang N, Yan H, Chen L, Gao J, et al. Scalp acupuncture enhances local brain regions functional activities and functional connections between cerebral hemispheres in acute ischemic stroke patients. *Anat Rec*. (2021) 304:2538–51. doi: 10.1002/ar.24746

16. Zhang J, Lu C, Wu X, Nie D, Yu H. Neuroplasticity of acupuncture for stroke: an evidence-based review of MRI. *Neural Plast*. (2021) 2021:2662585. doi: 10.1155/2021/2662585

17. Wang YF, Chen WY, Lee CT, Shen YY, Lan CC, Liu GT, et al. Combinations of scalp acupuncture location for the treatment of post-stroke hemiparesis: a systematic review and Apriori algorithm-based association rule analysis. *Front Neurosci*. (2022) 16:956854. doi: 10.3389/fnins.2022.956854

18. Yan XK, Wang FC, Wyang HF, Zhang Y, Yu L, Yang B. Perspective on the national standard standardized manipulation of acupuncture and moxibustion part II: scalp acupuncture. *Zhongguo Zhen Jiu*. (2009) 29:1001–4.

19. Groenewegen HJ. The basal ganglia and motor control. *Neural Plast*. (2003) 10:107–20. doi: 10.1155/NP.2003.107

20. Wang W, Zhao Y, Zhou L, et al. Oxygen level dependent functional magnetic resonance imaging and diffusion tensor imaging study on the mechanism of acupuncture of inferior nerve in cerebral infarction patients with hemiplegia. *Chin J Phys Med Rehabil*. (2015) 37:662–7.

21. Dong P, Cui FY, Tan ZJ, Jiang GD, Zhang H, Zou YH. A research of functional magnetic resonance imaging on the effects of acupuncture on brain reorganization in cerebral infarction patients with hemiplegia at spasm stage. *Chin J Rehabil Med*. (2010) 25:507–13.

22. Fang S-h, Zhang S-z, Liu H. Study on brain response to acupuncture by functional magnetic resonance imaging - observation on 14 healthy subjects. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. (2006) 26:965–8.

23. You YN, Song MY, Park GC, Na CS, Han JY, Cho MR. Meta-analysis on randomized controlled trials for scalp acupuncture treatment of stroke: a systematic review. *J Tradit Chin Med*. (2018) 38:465–79. doi: 10.1016/S0254-6272(18)30879-3

24. Gillebert CR, Mantini D. Functional connectivity in the normal and injured brain. *Neuroscientist*. (2013) 19:509–22. doi: 10.1177/1073858412463168

25. Zhang Y, Liu H, Wang L, Yang J, Yan R, Zhang J, et al. Relationship between functional connectivity and motor function assessment in stroke patients with hemiplegia: a resting-state functional MRI study. *Neuroradiology*. (2016) 58:503–11. doi: 10.1007/s00234-016-1646-5

26. Park CH, Chang WH, Ohn SH, Kim ST, Bang OY, Pascual-Leone A, et al. Longitudinal changes of resting-state functional connectivity during motor recovery after stroke. *Stroke*. (2011) 42:1357–62. doi: 10.1161/STROKEAHA.110.596155

27. Tanaka S, Honda M, Sadato N. Modality-specific cognitive function of medial and lateral human Brodmann area 6. *J Neurosci*. (2005) 25:496–501. doi: 10.1523/JNEUROSCI.4324-04.2005

28. Wang ZM, Shan Y, Zhang M, Wei PH Li QG, Yin YY, Lu J. Projections of Brodmann area 6 to the pyramidal tract in humans: quantifications using high angular resolution data. *Front Neural Circuits*. (2019) 13:62. doi: 10.3389/fncir.2019.00062

29. Cirstea CM, Brooks WM, Craciunas SC, Popescu EA, Choi IY, Lee P, et al. Primary motor cortex in stroke: a functional MRI-guided proton MR spectroscopic study. *Stroke.* (2011) 42:1004–9. doi: 10.1161/STROKEAHA.110.601047

30. Volz LJ, Vollmer M, Michely J, Fink GR, Rothwell JC, Grefkes C. Time-dependent functional role of the contralesional motor cortex after stroke. *Neuroimage Clin.* (2017) 16:165–74. doi: 10.1016/j.nicl.2017.07.024

31. Wu P, Zeng F, Yin C, Xiong Y, Bai Y, Wang D, et al. Effect of acupuncture plus conventional treatment on brain activity in ischemic stroke patients: a regional homogeneity analysis. *J Tradit Chin Med.* (2017) 37:650–8. doi: 10.1016/S0254-6272(17)30319-9

32. Casula EP, Pellicciari MC, Bonnì S, Spanò B, Ponzo V, Salsano I, et al. Evidence for interhemispheric imbalance in stroke patients as revealed by combining transcranial magnetic stimulation and electroencephalography. *Hum Brain Mapp.* (2021) 42:1343–58. doi: 10.1002/hbm.25297

33. Shimizu T, Hosaki A, Hino T, Sato M, Komori T, Hirai S, Rossini PM. Motor cortical disinhibition in the unaffected hemisphere after unilateral cortical stroke. *Brain.* (2002) 125 (Pt 8):1896–907. doi: 10.1093/brain/awf183

34. Wang P, Zang Z, Zhang M, Cao Y, Zhao Z, Shan Y, et al. Longitudinal changes of sensorimotor resting-state functional connectivity differentiate between patients with thalamic infarction and pontine infarction. *Neural Plast.* (2021) 2021:7031178. doi: 10.1155/2021/7031178

35. Kropf E, Syan SK, Minuzzi L, Frey BN. From anatomy to function: the role of the somatosensory cortex in emotional regulation. *Braz J Psychiatry.* (2019) 41:261–9. doi: 10.1590/1516-4446-2018-0183

36. Uddin LQ, Nomi JS, Hébert-Seropian B, Ghaziri J, Boucher O. Structure and function of the human insula. *J Clin Neurophysiol.* (2017) 34:300–6. doi: 10.1097/WNP.0000000000000377

frontiers | Frontiers in Neurology

TYPE Systematic Review
PUBLISHED 26 May 2023
DOI 10.3389/fneur.2023.1099012

OPEN ACCESS

EDITED BY
Yang Ye,
Peking University Third Hospital, China

REVIEWED BY
Rita Cardoso,
Campus Neurológico Sénior (CNS), Portugal
Qiuning Liu,
China Academy of Chinese Medical Sciences, China

*CORRESPONDENCE
Weihong Li
✉ lwh@cdutcm.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 15 November 2022
ACCEPTED 15 May 2023
PUBLISHED 26 May 2023

CITATION
Wu J, Wang Y, Wang X, Xie Y and Li W (2023) A systematic review and meta-analysis of acupuncture in Parkinson's disease with dysphagia. *Front. Neurol.* 14:1099012. doi: 10.3389/fneur.2023.1099012

A systematic review and meta-analysis of acupuncture in Parkinson's disease with dysphagia

Jing Wu[1†], Yi Wang[1†], Xueyan Wang[2], Yujia Xie[1] and Weihong Li[1*]

[1]Basic Medical School of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Clinical Medical School of Chengdu University of Traditional Chinese Medicine, Chengdu, China

Objective: The systematic review and meta-analysis aimed to comprehensively evaluate acupuncture's efficacy and safety in treating dysphagia in Parkinson's disease (PD).

Methods: We searched PubMed, Cochrane Library, Embase, Web of Science, China Knowledge Infrastructure (CNKI), China Science Journal Database (VIP), Wan-fang Database, and the China Biomedical Literature Service System (CBM) for randomized controlled trials (RCTs) comparing the efficacy of acupuncture alone or in combination with control treatment in improving dysphagia by October 2022. The degree of dysphagia was the primary outcome indicator, with secondary outcomes including serum albumin (ALB) and hemoglobin (Hb) levels, the incidence of pneumonia, and adverse events. Two investigators independently extracted information according to the inclusion and exclusion criteria. Data synthesis was calculated by RevMan (V.5.4.1) software.

Results: This study included ten randomized controlled trials with 724 patients. Most RCTs have a high or uncertain risk of bias due to the lack of a blinded design. Meta-analysis showed that acupuncture combined with control treatment was superior to control treatment alone in improving Videofluoroscopic Swallowing Study (VFSS) scores (MD: 1.48; 95% CI: 1.16, 1.81; $P < 0.00001$) and reducing Standardized Swallowing Assessment (SSA) scores (MD: −3.08; 95% CI: −4.01, −2.15; $P < 0.00001$). Acupuncture combined with control therapy has a more significant benefit in improving the clinical efficiency of dysphagia in PD (RR: 1.40; 95%CI: 1.25, 1.58; $P < 0.00001$). Compared to the control group without acupuncture, acupuncture improved the nutritional status of patients and increased their serum ALB (MD: 3.38, 95%CI: 1.83, 4.92, $P < 0.00001$) and Hb levels (MD: 7.66; 95%CI: 5.57, 9.75; $P < 0.00001$). Three RCTs reported that the rate of pulmonary infections in the acupuncture group was lower than without acupuncture intervention (RR: 0.29, 95% CI: 0.14, 0.63; $P = 0.001$).

Conclusion: Acupuncture could be recommended as an adjunctive treatment for dysphagia in PD. However, due to the high risk of bias in the included studies, more high-quality evidence is needed to confirm the efficacy and safety of acupuncture for dysphagia in PD.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022370221.

KEYWORDS

acupuncture, Parkinson's disease, dysphagia, systematic review, meta-analysis

1. Introduction

Dysphagia is a highly associated non-motor symptom of Parkinson's disease (PD), attributed to autonomic and gastrointestinal dysfunction (1, 2). However, it is only in the last few years that the importance of dysphagia has been recognized and has become a hot topic of research (3). Dysphagia can appear at any point during Parkinson's disease (4, 5). The prevalence of dysphagia in PD ranges from 11 to 81%, depending on the disease stage, disease course, or assessment method (6). Swallowing disorders adversely affect the diet and medication intake of PD patients, making nutritional intake and medication efficacy not guaranteed, reducing patients' quality of life, and in severe cases, even pneumonia and asphyxia (7, 8). In particular, aspiration pneumonia due to swallowing disorders is one of the leading causes of death in all patients with PD syndrome (9, 10). In addition, patients with PD with dysphagia have a higher prevalence of affective symptoms such as fear and depression (11, 12). Therefore, treating dysphagia in patients with PD is of clinical importance.

The mechanism of dysphagia in PD is unclear and involves both dopaminergic and non-dopaminergic (13). Dopaminergic treatment is known to improve motor and pulmonary function in Parkinson's patients; however, the effect of dopamine on swallowing function remains controversial (14–18). As dysphagia often aggravates the progression of PD, compensatory and rehabilitative strategies are commonly used to maintain functional swallowing, minimize the incidence and mortality of malnutrition and pulmonary infection, and maintain a satisfactory quality of life (19, 20). The short-term effects of Compensatory strategies such as changing eating habits, adjusting swallowing posture, and swallowing training are significant, but the long-term consequences may not be immediate (21–24).

Acupuncture is a traditional treatment in China, characterized by simple operation and easy acceptance by patients. The efficacy of acupuncture has been clinically verified, widely used in treating PD worldwide (25, 26), and included in the expert consensus on dysphagia treatment in China (27). It has been confirmed by clinical research and systematic review that acupuncture treatment has a good effect in improving the symptoms of Parkinson's disease patients with dysphagia, reducing adverse reactions of drugs, and improving the quality of life of patients, and has attracted more and more attention (28, 29). However, the effectiveness of acupuncture for treating dysphagia in patients with PD has not been fully confirmed due to the lack of highly credible evidence. Therefore, we designed this meta-analysis to review and evaluate the effects of acupuncture on swallowing function in patients with PD, aiming to provide a reference for clinical treatment.

2. Methods

This systematic review was developed based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) and checked by the PRISMA checklist (Appendix 1). The method used in this systematic review has been previously registered in PROSPERO (CRD42022370221), which is available from https://www.crd.york.ac.uk/prospero/.

2.1. Data sources and search strategy

From the establishment of the database to October 2022, we searched four English databases (PubMed, Cochrane Library, Embase, Web of Science) and four Chinese databases [China Knowledge Infrastructure (CNKI), China Science Journal Database (VIP), Wan-fang Database, and China Biomedical Literature Service System (CBM)]. No restrictions on countries or types of articles. The search terms included Parkinson's disease, Parkinson's disorders, deglutition disorders, dysphagia, and acupuncture, and the specific search strategy is shown in Appendix 2. In addition, we manually searched references cited in the included studies, previously published systematic reviews, and others to make sure that no literature was missed.

2.2. Inclusion and exclusion criteria

According to the PICOS principles, the inclusion criteria for this study were as follows: (1) Participants: Patients with a definite diagnosis of Parkinson's disease and tested for swallowing function, with dysphagia as a clinical manifestation of difficulty eating or choking on water. The diagnostic criteria for PD refer to the Chinese Guidelines for Diagnosis and Treatment of Parkinson's Disease (2016 Revision) and the diagnostic criteria for PD formulated by the Movement Disorder Society in 2015 (30, 31). There are no restrictions on age, gender, course of the disease, race, etc. (2) Interventions: The experimental group received acupuncture as a stand-alone or adjunctive treatment. All methods of treating conditions by stabbing needles into patients according to acupoints and using acupuncture techniques are considered acupuncture therapy, including general acupuncture, electroacupuncture, warming acupuncture, thumbtack needle, neck needling, prick bleeding, etc. There is no restriction on the specific intervention time, acupuncture point, and treatment course. (3) Control: The control group may use conventional therapy, swallowing rehabilitation, sham acupuncture, neuromuscular electrical stimulation, and so on. (4) Outcomes: The degree of dysphagia is the primary outcome indicator. Swallowing function can be assessed by the videofluoroscopic swallowing study (VFSS) (the higher the score, the better the swallowing function), the standardized swallowing assessment scale (SSA) (the lower the score, the better the swallowing function), and the water swallow test. Secondary outcomes included serum albumin (ALB) and hemoglobin (Hb) levels, the incidence of pneumonia, and adverse events (AE). (5) Study type: Only randomized controlled trials (RCTs) were included.

Exclusion criteria were: (1) Previous dysphagia caused by stroke, malignant disease of the posterior pharynx, digestive tract diseases, etc. (2) Studies with unclear diagnostic or assessment criteria. (3) Acupuncture is combined with other Chinese medical methods (e.g., herbal medicine, tui na, acupressure, and others) to treat dysphagia. (4) The control group used Chinese medicine. (5) Duplicate published studies or studies

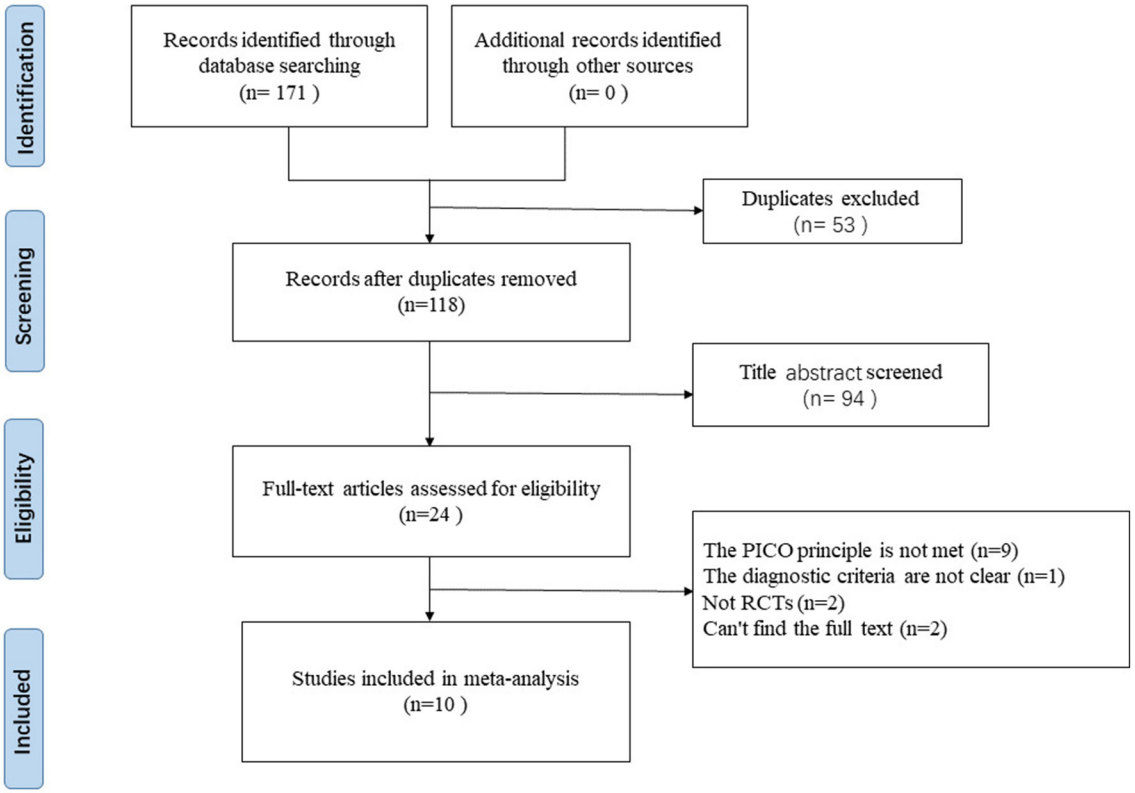


FIGURE 1
Flow diagram of searching and articles selection.

with incomplete data that remain unavailable after contacting the original author.

2.3. Data screening and extraction

All included studies were imported into Endnote 20. Two professionally trained reviewers (Jing Wu and Yi Wang) examined all studies separately, excluding duplicate articles and those that did not meet the inclusion criteria and finally identifying studies that met the intended inclusion criteria. After extracting data on authors, year of publication, age, sample size, duration of disease, intervention method, acupuncture points, outcome indicators, and adverse effects, the two reviewers cross-checked to ensure the accuracy of the data. Any disagreements during the screening and data extraction process could be resolved with the assistance of a third assessor (Yu-jia Xie). For literature lacking information, the original authors were contacted for additions.

2.4. Risk of bias

Two reviewers (Jing Wu and Yi Wang) independently performed the risk of bias assessments according to the Cochrane Handbook for Systematic Reviews of Interventions (32). The evaluation consisted of 7 entries: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective reporting, and other sources of bias. Each entry was assessed by the assessors and classified as "low risk," "high risk," or "uncertain."

2.5. Data analysis

RevMan 5.4.1 provided by Cochrane Collaboration was used for data analysis. Relative risk (RR) was chosen as the statistic for dichotomous data, and mean difference (MD) as the effect indicator for continuous variables to obtain *P*-values and 95% confidence intervals (CI). We defined $P < 0.05$ as a statistically significant difference. When the heterogeneity test is performed, the I^2 test is executed first and combined with quantified by the I^2 statistic for evaluation. If the heterogeneity test result is $I^2 < 50\%$, there is no significant heterogeneity among the results, and the fixed effect model is used for data analysis. If $I^2 \geq 50\%$, there is statistical heterogeneity among the results. After excluding apparent clinical and methodological heterogeneity, the random effect model was used for meta-analysis.

TABLE 1 Characteristics of included studies.

References	Sample size (T/C)	Age (mean ± SD)	Disease duration	Invention	Duration of treatment	Acupoints	Control	Outcome
Zhao et al. (33)	30/28	C: 36–70 T: 40–69	N/A	WA + FT	30 min each day	YaMen (DU15), LianQuan (RN23), JuQuan (EX-HN10), FengChi (GB20), RenZhong (DU26), NeiGuan (PC6), ZuSanLi (ST36)	FT	①
Li et al. (34)	43/43	C: 58.46 ± 4.3 T: 59.37 ± 4.89	C: (6.01 ± 1.25) y T: (5.80 ± 1.43) y	EA + FT	5 times a week for 30 min, 4 weeks	LianQuan (RN23), YiFeng (SJ17), FengChi (GB20), WanGu (GB12), WaiYuYe, WaiJinJin	FT	①②④⑤
Miu (35)	28/28	C: 67.88 ± 8.53 T: 67.50 ± 9.70	C: (5.23 ± 2.14) y T: (5.25 ± 2.67) y	A + P + FT	Once a day, 4 weeeks.	LianQuan (RN23), ShangLianquan, JinJin YuYe (EX-HN12)	FT	①④⑤⑥
Shi (36)	56/56	C: 55.52 ± 1.14 T: 65.58 ± 1.16	C: (4.52 ± 0.26) w T: (4.54 ± 0.24) w	WA + FT	30 min each day	RenZhong (DU26), YaMen (DU15), LianQuan (RN23), NeiGuan (PC6), FengChi (GB20)	FT	①⑥
Wang et al. (37)	20/20	C: 52–70 T: 50–72	C: (228 ± 136) d T: (234 ± 140) d	A + P + FT	30 min each day, 20–30 days	SheJian, JinJin YuYe (EX-HN12), YanHouBi, BaiHui (DU20), LianQuan (RN23), HeGu (LI4), Quchi (LI11), WaiGuan (SJ5), TaiChong (LR3), ZuSanLi (ST36), SanYinJiao (SP6); point selection by syndrome differentiation	FT	①
Wang (38)	45/45	C: 59 ± 10 T: 59 ± 10	C: (5.26 ± 1.02) y T: (5.31 ± 1.08) y	A + P + FT	5 times a week for 30 min, 4 weeks	ShenTing (DU24), BaiHui (DU20), ShangLianQuan, YinTang (DU29), TianZhu (BL10), FengChi (GB20), WaiGuan (SJ5), JinJin YuYe (EX-HN12), ZhaoHai (KI6), LieQue (LU7), YanHouBi	FT	①②
Wu et al. (29)	28/28	C: 65 ± 7 T: 63 ± 10	C: (5.4 ± 3.2) y T: (5.2 ± 3.3) y	A + P + FT	5 times a week for 30 min, 6 weeks	LianQuan (RN23), ShangLianQuan, FengChi (GB20), WaiGuan (SJ5), FengFu (DU16), YaMen (DU15), NeDaYing, JinJin YuYe (EX-HN12), YanHouBi	FT	①②④⑤
Wang et al. (39)	60/60	C: 52.0 ± 11 T: 54.0 ± 9.2	C: (1–2) y T: (1–2) y	A + FT	6 times a week for 30 min, 4 weeks	FengChi (GB20), YiMing (EX-HN13), GongXue, TunYan, LianQuan (RN23), WaiYuYe, WaJinJin,	FT	②③
Yin et al. (40)	30/30	C: 65 ± 5.25 T: 63.17 ± 5.02	C: (4.85 ± 5.40) y T: (4.60 ± 5.65) y	TN + FT	Once every 2 days for 24 h each time	LianQuan (RN23), YiFeng (SJ17), JiaLianQuan, JiaJiXue (C3, C4, C5)	FT + SM	①③
Xie (41)	22/24	C: 64.8 ± 5.5 T: 65.3 ± 5.4	C: (6.26 ± 1.62) y T: (6.28 ± 1.50) y	A + M	6 times a week for 30 min, 4 weeeks	TaiXi (KI3), ZhaoHai (KI6), BaiHui (DU20), GuanYuan (RN4), SanYinJiao (SP6), TaiChong (LR3), HeGu (LI4), FengChi (GB20)XueHai (SP10), LianQuan (RN23), PangLianQuan	NMES + M	②③⑥⑦

C, Control; T, Treatment; y, year; d, day; w, week; A, Acupuncture; WA, Warming acupuncture; TN, Thumbtack needle; P, Prick bleeding; FT, Functional training; SM, Sham acupuncture; M, Medicine; NMES, Neuromuscular electrical stimulation; N/A, Not applicable. ①, Water swallow test (WST); ②, Videofluoroscopic Swallowing Study (VFSS) scores; ③, Standardized Swallowing Assessment (SSA) scores; ④, Albumin (ALB); ⑤, Hemoglobin (Hb); ⑥, Incidence of pulmonary infection; ⑦, adverse events.

10.3389/fneur.2023.1099012

2.6. Subgroup analysis and sensitivity analysis

We considered that different types of acupuncture may have influenced the effectiveness of acupuncture, so we performed a subgroup analysis of the efficiency of varying needle types for treating dysphagia in PD. *P*-values $\geq$ 0.05 for the interaction indicated that the treatment effect did not differ significantly between subgroups. Sensitivity analysis was conducted when necessary.

3. Results

3.1. Literature selection

We searched a total of 171 papers from electronic databases. After excluding duplicate studies, 117 relevant studies were screened out. After reading the titles and abstracts of these studies, 24 relevant studies were identified. We read the full text before two independent reviewers performed further eligibility screening based on inclusion and exclusion criteria. The final 10 studies were included in the meta-analysis. The detailed literature screening process is shown in Figure 1.

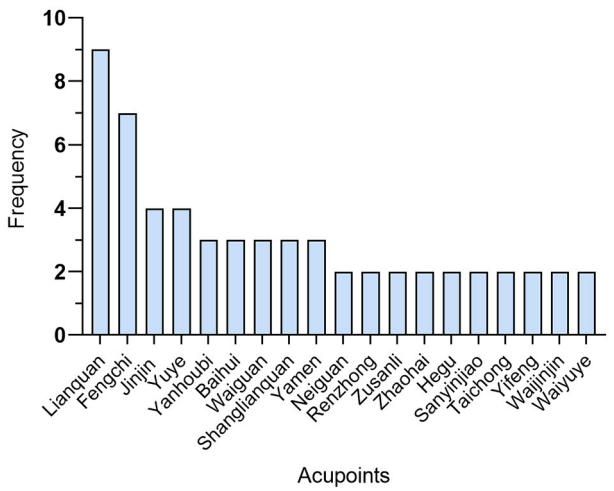


FIGURE 2
Frequency of commonly used acupoints.

3.2. Characteristics of the included literature

The 10 articles included in this study were all single-center randomized controlled trials conducted in China with 724 patients (362 in the experimental group and 362 in the control group) (29, 33–41). Two included literatures were master's theses (35, 41), and the remaining eight were journaled articles. The participants' ages ranged from 36 to 80 years. The average duration of dysphagia in PD was more than 1 year in seven of the 10 studies (29, 34, 35, 38–41), <1 year in two studies (36, 37), and no duration was mentioned in the remaining studies. Among all included studies, eight studies compared acupuncture plus conventional management (CM) with CM alone (including Western medicine, swallowing training, and oral sensorimotor training) (29, 33–39), one study compared acupuncture plus CM with sham acupuncture plus CM (40), and one study compared acupuncture plus Western medicine with neuromuscular electrical stimulation (NMES) plus Western medicine (41). The videofluoroscopic swallowing study (VFSS) is the "gold standard" for measuring the function of swallowing (10). Five studies used the VFSS to evaluate patients' swallowing function (29, 34, 38, 39, 41). In these five studies, one study recorded the time parameters of the patient's intake of paste and liquids (29), one study recorded the time parameters of the patient's input of paste (41), and three studies recorded the total VFSS score using the penetration/aspiration scale (34, 38, 39). In addition, three studies used the standardized swallowing assessment scale (SSA) (39–41). Eight studies performed the water swallow test (29, 33–38, 40, 41), but one study had a different type of data than the others (38); one study was evaluated on various

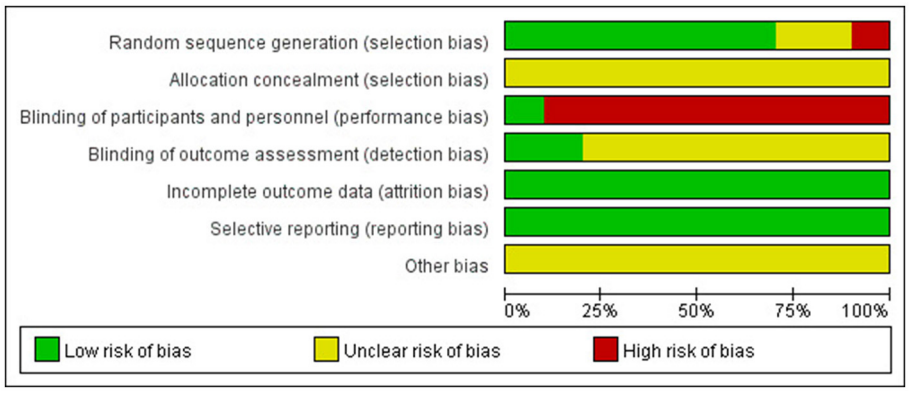


FIGURE 3
Risk of bias graph.

standards (34). For secondary outcomes, three studies (29, 34, 35) measured serum ALB and Hb levels reflecting nutritional status, three studies (35, 36, 41) documented the incidence of pulmonary infections after treatment, and one study (41) reported the safety of acupuncture. The specific characteristics of the included studies are shown in Table 1.

3.3. Acupuncture protocols included in the literature

Among the 10 included studies, warming acupuncture was used in two studies (33, 36), electroacupuncture was used in one study (34), manual acupuncture was used in two studies (38, 40), thumbtack needle was used in one study (40), and manual acupuncture combined with prick bleeding was used in four studies (29, 35, 37, 38). All the included literature described the selection of acupoints, as shown in Figure 2. Commonly used acupoints include Lianquan, Fengchi, Yamen, Baihui, Wangu, Jinjin, and Yuye. The needle retention time of body acupuncture is 30 min, and that of the intradermal needle is 24 h. Jinjin, Yuye, and Yanhoubi were punctured for bleeding without needle retention. The treatment frequency of acupuncture was once a day or every other day. The treatment period ranged from 20 days to 6 weeks. In all the studies, only two studies (37, 41) were treated based on syndrome differentiation, and the remaining studies applied fixed-point protocols. Of the included studies, only one study (35) provided information about acupuncturist certification, and eight studies (33–38, 40, 41) emphasized the sensation of De qi.

3.4. Risk of bias in the included literature

We assessed the risk of bias for all included articles. Two studies (35, 36) did not report a specific method of randomization, one study (33) used a randomization method with a high risk of bias according to visit order, and seven studies (29, 34, 37–41) reported the use of a random number table. None of the studies described allocation concealment and were judged to have an unclear risk of bias. Due to the specificity of acupuncture, only one study (40) told blinding patients to the use of sham acupuncture; the other studies did not mention the blinded design, which should be considered a high risk of bias. Two studies (39, 41) were blinded to the outcome indicator measure. All included RCTS had a low risk of bias in data completeness and selective reporting. The risk of bias assessment is summarized in Figures 3, 4.

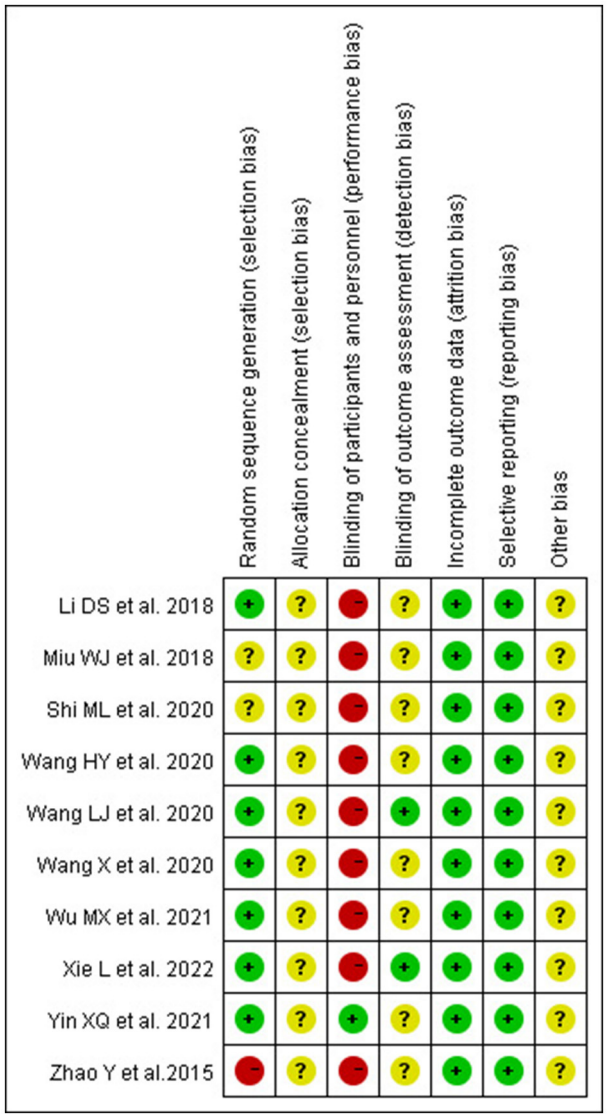


FIGURE 4
Risk of bias summary.

3.5. Results of the meta-analysis

3.5.1. VFSS scores

Five studies reported post-treatment VFSS scores (29, 34, 38, 39, 41), but we included only three studies due to differences in measurement methods across research (34, 38, 39). There was no significant heterogeneity between the three RCTs ($P = 0.38$, $I^2 = 0\%$), and the fixed-effects model showed a meaningful difference in VFSS scores between acupuncture combined with rehabilitation training (RT) and RT alone (MD: 1.48; 95% CI: 1.16, 1.81; $P < 0.00001$), indicating that acupuncture assisted treatment can significantly improve dysphagia in patients with PD, as shown in Figure 5.

3.5.2. SSA scores

SSA scores were reported in three studies (39–41). Since included studies showed no considerable heterogeneity ($P = 0.65$, $I^2 = 0\%$), fixed-effects models were used for analysis. The results showed that swallowing function was better in the acupuncture-treated group compared with the control group without acupuncture (MD: −3.08; 95% CI: −4.01, −2.15; $P < 0.00001$). See Figure 6.

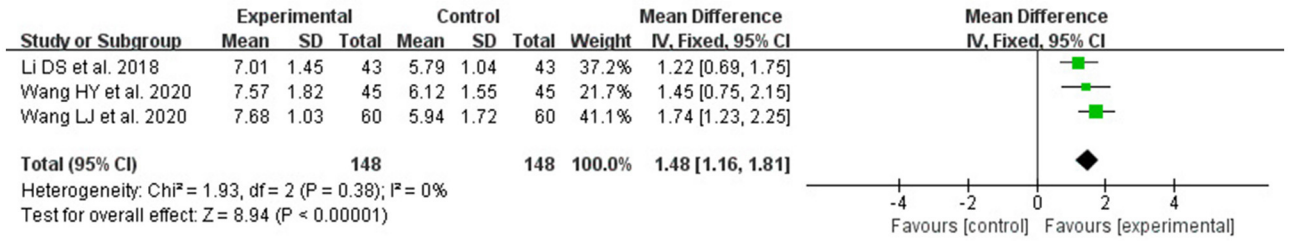


FIGURE 5
Forest plot of VFSS scores comparison between acupuncture and control group.

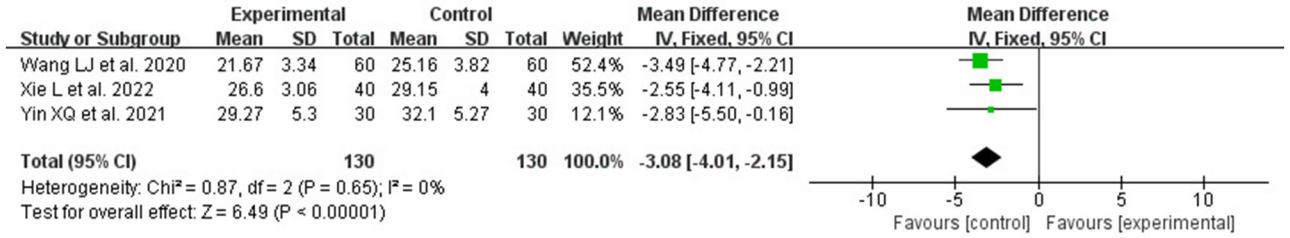


FIGURE 6
Forest plot of SSA scores comparison between acupuncture and control group.

3.5.3. The efficiency of the water swallow test

According to the grading and quantization standards determined in the Water swallow test (WST), the improvement of swallowing function was divided into four grades in six studies (29, 33, 35–37, 40), namely, cure: after treatment, the patient's swallowing disorder completely disappeared, the WST results rose to Grade I (swallow the water smoothly in one go), and there were no other discomfort symptoms; Remarkable effect: after treatment, the patient's dysphagia disappeared, and the WST results rose to Grade I–II (swallow without choking in two or more times, without other discomfort symptoms); Effective: After treatment, the patient's dysphagia has been improved, and the WST is Grade II–III (swallow in one go but with choking), with slight discomfort; Ineffective: The swallowing disorder did not improve or even worsen after treatment, and the WST result was higher than Grade III (choked frequently or could not swallow it all). The total effective rate is the sum of the number of cured, remarkable effects, and influential people as a percentage of the total number of people. As there was no significant heterogeneity between these studies, a meta-analysis was performed using a fixed-effects model ($P = 0.58$, $I^2 = 0\%$). The results showed that patients who received acupuncture combined with RT had more improvement in swallowing function compared with RT alone (RR: 1.40; 95% CI: 1.25, 1.58; $P < 0.00001$; Figure 7).

We divided the included studies into two subgroups according to the type of acupuncture to discuss the efficacy of acupuncture due to the variety of acupuncture methods in the experimental group. As shown in Figure 8, compared with RT alone, warming acupuncture plus RT (2 studies, RR: 1.41; 95% CI: 1.19, 1.66; $P < 0.0001$; heterogeneity: $I^2 = 23\%$, $P = 0.26$), prick bleeding plus rehabilitation (3 studies, RR: 1.33; 95% CI: 1.10, 1.60; $P = 0.003$; heterogeneity: $I^2 = 0\%$, $P = 0.53$) and thumbtack needle plus RT (1 study, RR: 1.63; 95% CI: 1.13, 2.34; $P = 0.009$;) both significantly enhanced the effective rate.

3.5.4. Nutritional status

The three included studies (29, 34, 35) assessed the nutritional status of the treatment and control groups through patient serum ALB and Hb levels. The statistical data showed that acupuncture combined with RT had a remarkable effect on ALB level (MD: 3.38, 95%CI:1.83, 4.92, $P < 0.00001$; heterogeneity: $I^2 = 82\%$, $P = 0.004$; Figure 9) and Hb level (MD: 7.66; 95% CI: 5.57, 9.75; $P < 0.00001$;f heterogeneity: $I^2 = 17\%$, $P = 0.30$; Figure 10) under conventional drug treatment, which indicated that acupuncture could improve the nutritional status of patients with dysphagia in PD.

3.5.5. Incidence of pulmonary infections

The incidence of pulmonary infection is reported in three articles (35, 36, 41). The results of the fixed-effect model analysis showed that the incidence of pneumonia in the acupuncture group was significantly lower than that in the non-acupuncture group (RR: 0.29, 95% CI: 0.14, 0.63, $P = 0.001$; heterogeneity: $I^2 = 0\%$, $P = 0.40$; Figure 11).

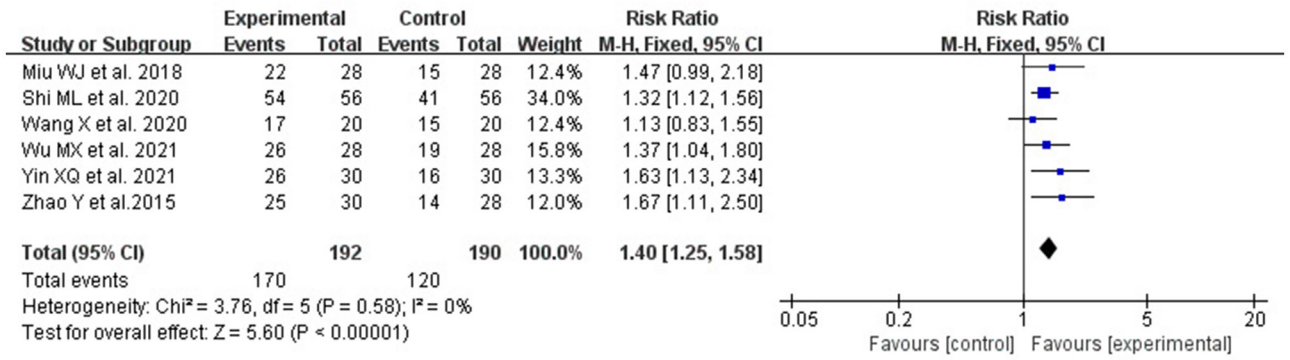


FIGURE 7
The forest plot shows a comparison of total efficiency rates between the acupuncture and the control group.

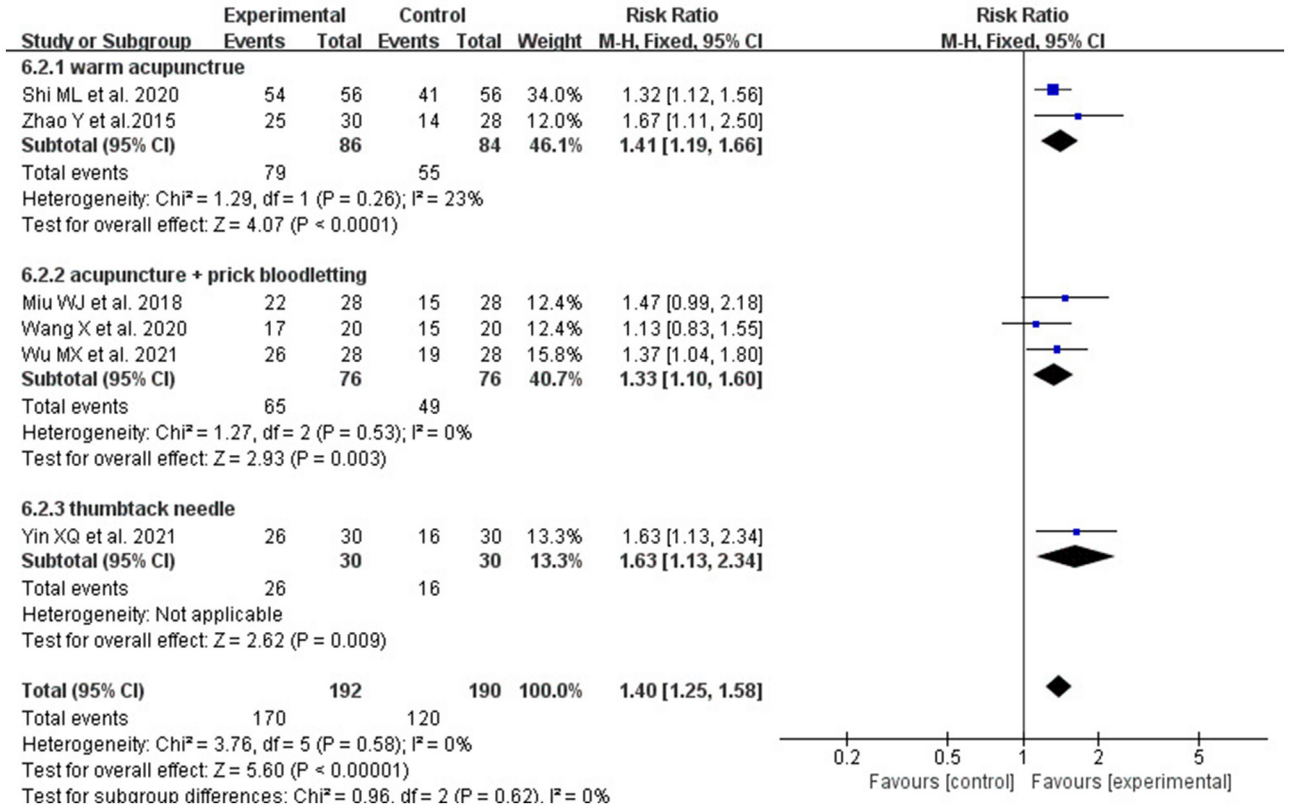


FIGURE 8
The forest plot shows a comparison of effectiveness in treating Parkinson's dysphagia between the acupuncture and the control group, based on a subgroup analysis of different acupuncture methods.

3.5.6. Adverse events

Of the ten included studies, only one reported no related acupuncture adverse events during the trial (41), and the remaining nine studies did not report adverse events.

3.6. Publication bias

Funnel plots were not used to investigate publication bias because of the limited number of included studies (<10 trials).

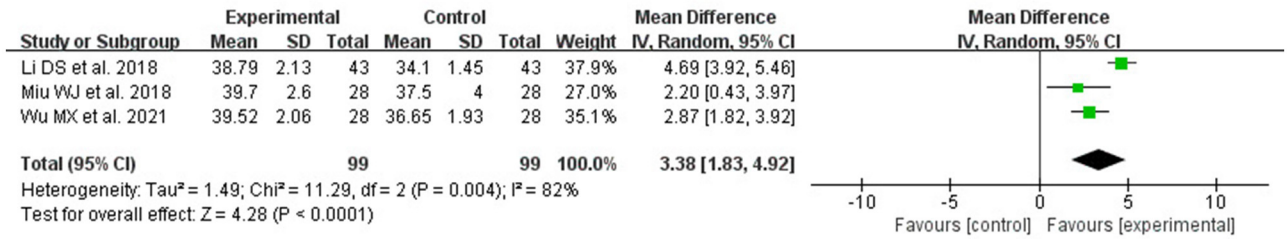


FIGURE 9
Forest plot of ALB level in comparison between acupuncture group and control.

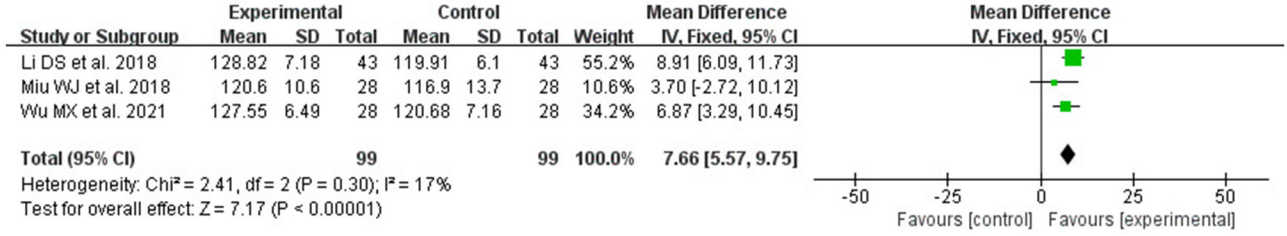


FIGURE 10
Forest plot of Hb level in comparison between acupuncture group and control.

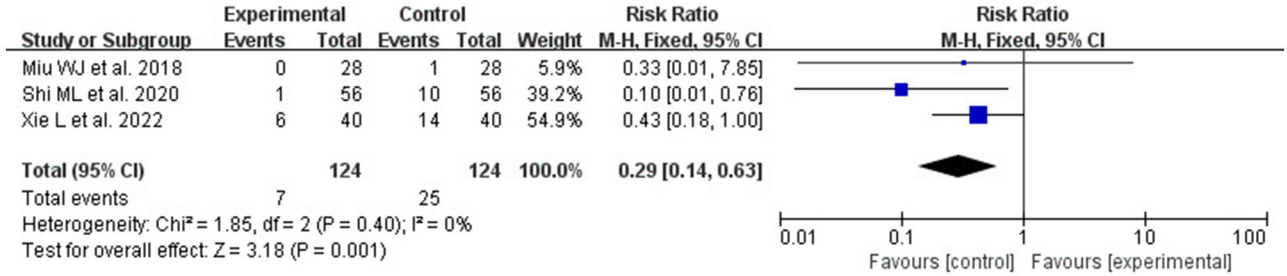


FIGURE 11
Forest plot comparing the incidence of pulmonary infection between the acupuncture and the control group.

4. Discussion

4.1. Main results and analysis

This systematic review included 10 RCTs that evaluated the patient's swallowing function, nutritional status, and incidence of pneumonia. Our study showed that acupuncture combined with rehabilitation training increased the effectiveness of improving swallowing function in patients with swallowing disorders in Parkinson's disease and could lead to significant improvement in the patient's swallowing function and reduced the incidence of pulmonary infections. In addition, patients with dysphagia are often accompanied by malnutrition, and the nutritional level is closely related to the prognosis of patients. The study on the correlation between dysphagia and the nutritional status of PD patients shows that the better the nutritional status of patients, the better the prognosis (42). ALB and Hb have the characteristics of convenient and rapid detection in the clinic, and are widely used to evaluate nutritional status. In malnourished patients, low albumin levels have been long-standing (43, 44). According to the results of Meta-analysis, even in the presence of chronic inflammation, several blood biomarkers including albumin, prealbumin and hemoglobin are useful biochemical indicators of adult malnutrition (45). When acupuncture is combined with the treatment measures of the control group, the serum ALB and Hb levels of patients are significantly improved. Thus, the results of this systematic review support acupuncture as an augmentation approach to improve dysphagia in Parkinson's disease. We did not obtain sufficient evidence regarding the effectiveness of acupuncture alone, which may be related to the synergistic effects of acupuncture with other therapies.

The Water Swallow Test and VFSS are commonly used clinical assessment methods of dysphagia, among which VFSS is

recognized as the gold standard for the diagnosis of dysphagia (46). However, it needs special equipment, requires the subject to have a certain physical strength, can cooperate with the examination, and is radioactive in operation, thus posing the risk of aspiration of contrast agent, which limits its clinical application to a certain extent. The most important complication of dysphagia is risk for aspiration, so the detection of misophagia is the main purpose of clinical evaluation. A positive assessment of SSA score may provide a preliminary indication that a patient may have swallowed incorrectly, but since this is only a preliminary assessment and screening, it is necessary to refer the patient to an experienced language therapist for reassessment and further examination to identify dysphagia, which is one of the reasons why only a small number of studies use the SSA scale (47, 48).

4.2. Mechanism of acupuncture

Dysphagia is a serious adverse factor in the prognosis of PD patients and a significant cause of death (49). It has been suggested that various pathological changes involving nerves and muscles during the progression of PD patients can lead to impaired neuromodulation at any level of the peripheral nerves, brainstem swallowing centers, cerebral cortex, and subcortical centers, resulting in PD dysphagia (50, 51). Autopsy reports from PD patients show that α -synuclein is present in the peripheral sensory nerves of dysphagia patients and in the motor nerves that dominate the pharyngeal muscles, compared to patients without dysphagia (49, 52). There is a belief that although the central or peripheral nervous system in PD patients can reorganize structurally or functionally, this remodeling function does not occur naturally and needs to be achieved by receiving stimulation (29). Some studies have shown acupuncture can stimulate the supraglottic and parasympathetic nerves, increase cerebral blood flow in patients, promote the repair and reconstruction of pharyngeal reflex arc function, and thus enhance swallowing function. (53–58). Acupuncture also enhances the excitability of the central nervous system, coordinates the fine movements of the tongue and pharynx, improves the paralysis of the pharyngeal muscles, and further improves dysphagia (59–62). Qi Ling et al. showed that electroacupuncture could reduce the content of α -synuclein by inhibiting neuritis reaction, slowing down the apoptosis rate of dopaminergic neurons in the substantia nigra, improving the dopaminergic pathway, and thus promoting swallowing function (62).

4.3. Clinical effects

"Where the acupoints are located, the indications are located" is one of the roles of acupoints. The ability of an acupoint to treat diseases in its location and the adjacent organs, tissues, and organs is a common feature of all acupoints (63). This article showed that acupuncture has good effect on swallowing function in patients with dysphagia in PD. Acupuncture is mainly taken from the posterior pharynx and head, and the commonly used acupoints are Lianquan, Fengchi, Jinjin, and Yuyi. Puncture and bloodletting of the posterior pharyngeal wall is also commonly used. Follow-up studies should further investigate and screen stationary and effective acupuncture points to form a fixed localized acupuncture treatment plan to benefit more patients with dysphagia in PD. "Syndrome differentiation" is the basic principle of TCM (64). Wang et al. (37) and Xie (41) selected different acupuncture points for various symptoms of the patients, fully reflecting the personalized treatment of TCM and the treatment policy of seeking the fundamental cause of the disease.

Overall, this study is the first systematic evaluation and meta-analysis of the effectiveness and safety of acupuncture for Parkinson's swallowing disorder. We hope to provide doctors with a range of treatment strategies and help them design individualized interventions. According to the above results, doctors can develop the most appropriate approach for dysphagia in PD based on proximal acupoint selection and Syndrome differentiation, combined with the proper acupuncture method.

4.4. Limitations

Although this study followed the criteria stated in PRISMA, there are still some limitations. First, even though the meta-analysis shows no obvious heterogeneity, all the included studies are single-center RCTs in China, and the small sample size and the diversity of treatment methods may lead to certain potential biases, thus affecting the reliability of the results. Second, due to the specific nature of acupuncture therapy, studies are difficult to implement, blinding for participants and personnel, and there is a high risk of bias. Third, as only one of the included studies mentioned the absence of adverse effects and none of the other studies reported on the adverse effects of acupuncture, there was no systematic review of the possible acupuncture problems during treatment. Hence, to more comprehensively and objectively evaluate the efficacy of acupuncture in treating dysphagia in Parkinson's disease, future studies need to raise the sample size, provide reasonable allocation concealment and blinded design for trials, and provide more comprehensive reference information for subsequent research studies.

5. Conclusion

Acupuncture is effective as a complementary therapy for dysphagia in PD, not only improving patients' swallowing function but also enhancing their nutritional status and reducing the incidence of pneumonia. However, due to the high risk of bias in the included studies, the results should be interpreted with caution, and multicenter, more rigorous, and high-quality RCTs are necessary for subsequent analyses.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JW and XW selected a topic for the study. WL revised the manuscript. JW and YW conducted data extraction and quality assessment, completed the data synthesis, drafted the manuscript, and performed the search strategy. YX arbitrated in cases of disagreement and ensured the absence of errors. All authors have developed the search strategy, read, and approved the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China, grant number: 81873204 and the Key Research and Development Program of Science and Technology of Sichuan Province of China, grant number: 2021YFS0040.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1099012/full#supplementary-material

References

1. Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol.* (2015) 14:625–39. doi: 10.1016/S1474-4422(15)00007-1

2. Warnecke T, Schäfer KH, Claus I, Del Tredici K, Jost WH. Gastrointestinal involvement in Parkinson's disease: pathophysiology, diagnosis, and management. *NPJ Parkinsons Dis.* (2022) 8:31. doi: 10.1038/s41531-022-00295-x

3. Simons JA. Swallowing dysfunctions in Parkinson's disease. *Int Rev Neurobiol.* (2017) 134:1207–38. doi: 10.1016/bs.irn.2017.05.026

4. Sung HY, Kim J-S, Lee K-S, Kim Y-I, Song I-U, Chung S-W, et al. The prevalence and patterns of pharyngoesophageal dysmotility in patients with early stage Parkinson's disease. *Mov Disord.* (2010) 25:2361–8. doi: 10.1002/mds.23290

5. Durcan R, Wiblin L, Lawson RA, Khoo TK, Yarnall AJ, Duncan GW, et al. Prevalence and duration of non–motor symptoms in prodromal Parkinson's disease. *Eur J Neurol.* (2019) 26:979–85. doi: 10.1111/ene.13919

6. Takizawa C, Gemmell E, Kenworthy J, Speyer R. A systematic review of the prevalence of oropharyngeal dysphagia in stroke, Parkinson's disease, Alzheimer's disease, head injury, and pneumonia. *Dysphagia.* (2016) 31:434–41. doi: 10.1007/s00455-016-9695-9

7. Lin CW, Chang YC, Chen WS, Chang K, Chang HY, Wang TG. Prolonged swallowing time in dysphagic Parkinsonism patients with aspiration pneumonia. *Arch Phys Med Rehabil.* (2012) 93:2080–4. doi: 10.1016/j.apmr.2012.07.010

8. Suttrup I, Warnecke T. Dysphagia in Parkinson's disease. *Dysphagia.* (2016) 31:24–32. doi: 10.1007/s00455-015-9671-9

9. Kwon M, Lee JH. Oro–Pharyngeal dysphagia in Parkinson's disease and related movement disorders. *J Mov Disord.* (2019) 12:152–60. doi: 10.14802/jmd.19048

10. Patel B, Legacy J, Hegland KW, Okun MS, Herndon NE. A comprehensive review of the diagnosis and treatment of Parkinson's disease dysphagia and aspiration. *Expert Rev Gastroenterol Hepatol.* (2020) 14:411–24. doi: 10.1080/17474124.2020.1769475

11. Dissanayaka NNW, Sellbach A, Matheson S, O'Sullivan JD, Silburn PA, Byrne GJ, et al. Anxiety disorders in Parkinson's disease: prevalence and risk factors. *Mov Disord.* (2010) 25:838–45. doi: 10.1002/mds.22833

12. Han M, Ohnishi H, Nonaka M, Yamauchi R, Hozuki T, Hayashi T, et al. Relationship between dysphagia and depressive states in patients with Parkinson's disease. *Parkinsonism Relat Disord.* (2011) 17:437–9. doi: 10.1016/j.parkreldis.2011.03.006

13. Bhidayasiri R, Phuenpathom W, Tan AH, Leta V, Phumphid S, Chaudhuri KR, et al. Management of dysphagia and gastroparesis in Parkinson's disease in real–world clinical practice–Balancing pharmacological and non–pharmacological approaches. *Front Aging Neurosci.* (2022) 14:979826. doi: 10.3389/fnagi.2022.979826

14. Herer B, Arnulf I, Housset B. Effects of levodopa on pulmonary function in Parkinson's disease. *Chest.* (2001) 119:387–93. doi: 10.1378/chest.119.2.387

15. LeWitt PA. Levodopa therapy for Parkinson's disease: pharmacokinetics and pharmacodynamics. *Mov Disord.* (2015) 30:64–72. doi: 10.1002/mds.26082

16. Olanow CW, Calabresi P, Obeso JA. Continuous dopaminergic stimulation as a treatment for Parkinson's disease: current status and future opportunities. *Mov Disord.* (2020) 35:1731–44. doi: 10.1002/mds.28215

17. Lim A, Leow L, Huckabee ML, Frampton C, Anderson T. A pilot study of respiration and swallowing integration in Parkinson's disease: "on" and "off" levodopa. *Dysphagia.* (2008) 23:76–81. doi: 10.1007/s00455-007-9100-9

18. Warnecke T, Suttrup I, Schröder JB, Osada N, Oelenberg S, Hamacher C, et al. Levodopa responsiveness of dysphagia in advanced Parkinson's disease and reliability testing of the FEES–Levodopa–test. *Parkinsonism Relat Disord.* (2016) 28:100–6. doi: 10.1016/j.parkreldis.2016.04.034

19. Umemoto G, Furuya H. Management of dysphagia in patients with Parkinson's disease and related disorders. *Intern Med.* (2020) 59:7–14. doi: 10.2169/internalmedicine.2373-18

20. Schindler A, Pizzorni N, Cereda E, Cosentino G, Avenali M, Montomoli C, et al. Consensus on treating dysphagia in Parkinson's disease. *J Neurol Sci.* (2021) 430:120008. doi: 10.1016/j.jns.2021.120008

21. Steele CM, Alsanei WA, Ayanikalath S, Barbon CE, Chen J, Cichero JA, et al. The influence of food texture and liquid consistency modification on swallowing physiology and function: a systematic review. *Dysphagia.* (2015) 30:2–26. doi: 10.1007/s00455-014-9578-x

22. Young JL, Macrae P, Anderson C, Taylor–Kamara I, Humbert IA. The sequence of swallowing events during the chin–down posture. *Am J Speech Lang Pathol.* (2015) 24:659–70. doi: 10.1044/2015_AJSLP-15-0004

23. Troche MS, Okun MS, Rosenbek JC, Musson N, Fernandez HH, Rodriguez R, et al. Aspiration and swallowing in Parkinson disease and rehabilitation with EMST: a randomized trial. *Neurology.* (2010) 75:1912–19. doi: 10.1212/WNL.0b013e3181fef115

24. Athukorala RP, Jones RD, Sella O, Huckabee ML. Skill training for swallowing rehabilitation in patients with Parkinson's disease. *Arch Phys Med Rehabil.* (2014) 95:1374–82. doi: 10.1016/j.apmr.2014.03.001

25. Wen X, Li K, Wen H, Wang Q, Wu, Z, Yao Z. Acupuncture–related therapies for Parkinson's disease: a meta–analysis and qualitative review. *Front Aging Neurosci.* (2021) 13:676827. doi: 10.3389/fnagi.2021.676827

26. Li X, Wei W, Wang Y, Wang Q, Liu Z. Global trend in the research and development of acupuncture treatment on Parkinson's disease from 2000 to 2021: a bibliometric analysis. *Front Neurol.* (2022) 13:906317. doi: 10.3389/fneur.2022.906317

27. China Expert Consensus on Assessment and Treatment of Swallowing Disorders (2017 Edition) [in Chinese]. *Chin J Phys Med Rehabil*. (2018) 40:1–10

28. Cai GY. *Systematic Review of Acupuncture Treatment for Dysphagia*. Heilongjiang: Heilongjiang University of Chinese Medicine (2021).

29. Wu MX, Wang LG, Li HP, Zeng X. Acupuncture adjuvant treatment for dysphagia in patients with Parkinson's disease: a randomized controlled trial [in Chinese]. *Zhongguo Zhen Jiu*. (2021) 41:485–8. doi: 10.13703/j.0255-2930.20200724-0002

30. Parkinson's Disease and movement Disorders Group, Neurology Branch of Chinese Medical Association, Department of Parkinson's Disease and movement Disorders, Neurology Branch of Chinese Medical Doctor Association. Diagnostic criteria of Parkinson's disease in China (2016 edition). *Chin J Neurol*. (2016) 49:268–271.

31. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria forParkinson's disease. *Mov Disord*. (2015) 30:1591–601. doi: 10.1002/mds.26424

32. Higgins JPT, *Green S, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*. London, UK: The Cochrane Collaboration (2011).

33. Zhao Y, Liu P, Wang M, Yin YY. Clinical observation of needle warming moxibustion combined with function training on dysphagia of Parkinson's disease. *Chin Med Modern Distance Educ China*. (2015) 13:61–3.

34. Li DS, Ma JJ, Li X, Ma YJ, Qi YW, Chen SY, et al. Efficacy of electroacupuncture combined with early swallowing training in the treatment of patients with Parkinson's disease with dysphagia. *Chin J Phys Med Rehabil*. (2018) 40:428–30.

35. Miu WJ. *Observation on Curative Effect of Acupuncture Combined with Rehabilitation Training in Treating Deglutition Disorder of Parkinson's Disease*. Zhejiang: Wenzhou Medical University (2018).

36. Shi LM. Effects of warm acupuncture and moxibustion combined with functional training on swallowing dysfunction in elderly patients with Parkinson's disease. *Healthful Friend*. (2020) 2020:113–4.

37. Wang X, Feng WX, Tian S, Li T, Li P, Liu ZB. Pharynx three-needle and tremor-ceasing eight-needle combined with rehabilitation training for dysphagia in Parkinson's disease. *Jilin J Chin Med*. (2020) 40:1383–6. doi: 10.13463/j.cnki.jlzyy.2020.10.035

38. Wang HY, Zuo GC, Liu J, Jiang Q, Wang S. Therapeutic observation of acupuncture combined with oral sensorimotor training for dysphagia in Parkinson disease. *Shanghai J Acu-Mox*. (2020) 39:440–5. doi: 10.13460/j.issn.1005-0957.2020.04.0440

39. Wang LJ, Wang X, Zhang JY, Li JS, Hou XL, Zhou LW. Effect of nape needle combined with deglutition training on deglutition disorder in Parkinson's disease. *Rehabil Med*. (2020) 30:202–5. doi: 10.3724/SP.J.1329.2020.03007

40. Yin XQ, Jin YX, Zheng XJ, Liu SN. Clinical study on thumbtack needle combined with rehabilitation training for dysphagia in Parkinson disease. *New Chin Med*. (2021) 53:172–5. doi: 10.13457/j.cnki.jncm.2021.06.044

41. Xie L. *Clinical Observation on Therapeutic Effect of Ziyin Qianyang Acupuncture Prescription of Dysphagia in Parkinson's Disease with Yin-Deficiency and Wind-Movement [in Chinese]*. Fujian: Fujian University of Traditional Chinese Medicine (2022).

42. Huan S, Yingying X, Mingming W, Huijie Y. Parkinson's patients with dysphagia and nutrition correlation. *China Sanit Eng*. (2021) 20:65–7. doi: 10.19937 / j.i SSN. 1671-4199.2021.01.025

43. Bernstein LH, Leukhardt–Fairfield CJ, Pleban W, Rudolph R. Usefulness of data on albumin and prealbumin concentrations in determining effectiveness of nutritional support. *Clin Chem*. (1989) 35:271–4. doi: 10.1093/clinchem/35.2.271

44. Lindor KD, Fleming CR, Ilstrup DM. Preoperative nutritional status and other factors that influence surgical outcome in patients with Crohn's disease. *Mayo Clin Proc*. (1985) 60:393–6. doi: 10.1016/S0025-6196(12)60849-0

45. Zhang Z, Pereira SL, Luo M, Matheson EM. Evaluation of blood biomarkers associated with risk of malnutrition in older adults: a systematic review and meta–analysis. *Nutrients*. (2017) 9:829. doi: 10.3390/nu9080829

46. Edmiaston J, Connor L Tabor, Ford AL. A simple bedside stroke dysphagia screen, validated against videofluoroscopy, detects dysphagia and aspiration with high sensitivity. *J Stroke Cerebrovasc Dis*. (2014) 23:712–6. doi: 10.1016/j.jstrokecerebrovasdis.2013.06.030

47. Yang EJ, Kim KW, Lim JY, Paik NJ. Relationship between dysphagia and mild cognitive impairment in a community–based elderly cohort: the Korean longitudinal study on health and aging. *J Am Geriatr Soc*. (2014) 62:40–6. doi: 10.1111/jgs.12606

48. Wu SL, Ma C, Huang FY, Yan TB. Clinical application of standardized swallowing assessment. *Chin J Phys Med Rehabil*. (2008) 30:396–9.

49. Wang P, Wang B, Chen XXong B, Xie F, Wu S, et al. Six–year follow–up of dysphagia in patients with Parkinson's disease. *Dysphagia*. (2022) 37:1271–8. doi: 10.1007/s00455-021-10387-0

50. Mu L, Sobotka S, Chen J, Su H, Sanders I, Adler CH, et al. Alpha–synuclein pathology and axonal degeneration of the peripheral motor nerves innervating pharyngeal muscles in Parkinson disease. *J Neuropathol Exp Neurol*. (2013) 72:119–29. doi: 10.1097/NEN.0b013e3182801cde

51. Monteiro L, Souza–Machado A, Pinho P, Sampaio M, Nóbrega AC, Melo A. Swallowing impairment and pulmonary dysfunction in Parkinson's disease: the silent threats. *J Neurol Sci*. (2014) 339:149–52. doi: 10.1016/j.jns.2014.02.004

52. Mu L, Sobotka S, Chen J, Su H, Sanders I, Nyirenda T, et al. Parkinson disease affects peripheral sensory nerves in the pharynx. *J Neuropathol Exp Neurol*. (2013) 72:614–23. doi: 10.1097/NEN.0b013e3182965886

53. Sun XQ, Zhou HF. lnvestigation of relevance between partial anatomy of Lianquan&Panglianquan and treatment of dysphagia. *J Liaoning Univ Trad Chin Med*. (2016) 18:147–9. doi: 10.13194/j.issn.1673-842x.2016.08.047

54. Meng YC, Wang C, Shang SQ, Ning LP, Zhou L, Han K. Effects of different acupuncture depths of Lianquan (CV 23) for dysphagia after stroke: a randomized controlled trial [in Chinese]. *Chin Acupunct Moxibust*. (2015) 35:990–4. doi: 10.4103/1673-5374.230272

55. Chang QY, Lin YW, Hsieh CL. Acupuncture and neuroregeneration in ischemic stroke. *Neural Regen Res*. (2018) 13:573–83.

56. Zhu W, Ye Y, Liu Y, Wang X-R, Shi G-X, Zhang S, et al. Mechanisms of acupuncture therapy for cerebral ischemia: an evidence–based review of clinical and animal studies on cerebral ischemia. *J Neuroimmune Pharmacol*. (2017) 12:575–92. doi: 10.1007/s11481-017-9747-4

57. Huang Y, Jiang X, Zhuo Y, Wik G. Complementary acupuncture in Parkinson's disease: a spect study. *Int J Neurosci*. (2010) 120:150–4. doi: 10.3109/00207450903316527

58. Takamoto K, Urakawa S, Sakai K, Ono T, Nishijo H. Effects of acupuncture needling with specific sensation on cerebral hemodynamics and autonomic nervous activity in humans. *Int Rev Neurobiol*. (2013) 111:25–48. doi: 10.1016/B978-0-12-411545-3.00002-X

59. Guo X, Ma T. Effects of acupuncture on neurological disease in clinical– and animal–based research. *Front Integr Neurosci*. (2019) 13:47. doi: 10.3389/fnint.2019.00047

60. Zhang H, Jiang YJ, Wei QL, Dong XL. Ecacy observation on body acupuncture and point pricking at Jinjin (EX-HN 12) and Yuye (EX-HN 13) for post-stroke dysphagia. *Chin Acupunct Moxibust*. (2013) 33:11–3. doi: 10.13703/j.0255-2930.2013.s1.006

61. Li ZH, Li HM, Ai NN. Clinical trials of Xingnao Kaiqiao needling combined with respiratory training for post–stroke dysphagia. *Zhen Ci Yan Jiu*. (2021) 46:875–9. doi: 10.13702/j.1000-0607.201011

62. Qi L, Wang Y, Li YN, Guo L, Ma J. Electroacupuncture improves behavioral activities by suppressing neuroinflammation and TLR4/NF-KB signaling in substantia nigra of midbrain in Parkinson' s disease rats. *Zhen Ci Yan Jiu*. (2021) 46:929–34+972. doi: 10.13702/j.1000-0607.200874

63. Li J. Discussion on the idea of proximal acupoint selection in the acupuncture and moxibustion treatment. *J Guangzhou Univ Trad Chin Med*. (2022) 39:124–8. doi: 10.13359/j.cnki.gzxbtcm.2022.01.022

64. Zhu J, Zhu XH. Discussion of TCM 'syndrome' and 'treatment based on syndrome differentiation'. *China J Trad Chin Med Pharm*. (2017) 32:21–4.

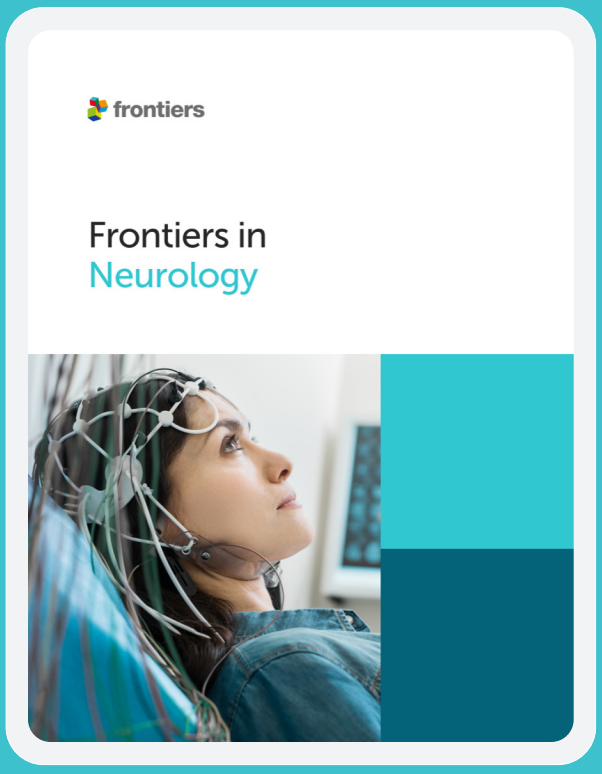
Frontiers in Neurology

Explores neurological illness to improve patient care

The third most-cited clinical neurology journal explores the diagnosis, causes, treatment, and public health aspects of neurological illnesses. Its ultimate aim is to inform improvements in patient care.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact