

# Effect of Acupuncture Combined with Butylphthalide Injection on Motor Function and Cognitive Function in Cerebral Infarction

NING LUO, LIANXIA ZOU<sup>1</sup>, KUI ZHAO<sup>2</sup> AND ZIKE LIU<sup>3\*</sup>

Department of Pharmacy, <sup>1</sup>Department of Traditional Chinese Medicine, Shenzhen Hospital of Beijing University of Chinese Medicine, Longgang, Shenzhen 518172, <sup>2</sup>Department of Pharmaceutics, Shenzhen People's Hospital, Longgang, Shenzhen 518020, <sup>3</sup>Department of Pharmaceutics, Shenzhen Hospital of Beijing University of Chinese Medicine, Longgang, Shenzhen 518172, China

## Luo *et al.*: Combined Effect of Acupuncture and Butylphthalide in Cerebral Infarction

To investigate the effect of acupuncture combined with butylphthalide injection on motor function and cognitive function and the expression of microRNAs in patients with cerebral infarction. 106 patients with acute cerebral infarction were randomly divided into control group and combined group. The patients in the control group were treated with routine and butylphthalide injection. The patients in the combined group were treated with acupuncture on the basis of the control group. Fugl Meyer scale was used to evaluate the changes of motor function before and after treatment. Mini mental state examination was used to evaluate the changes of cognitive function before and after treatment. Quantitative real-time polymerase chain reaction was used to detect the expression of microRNAs before and after treatment. After treatment, the Fugl Meyer scale score and total score of mini mental state examination scale of the two groups were significantly increased. Compared with the control group, the Fugl Meyer scale score and the total score of mini mental state examination scale in the combined group were significantly higher after treatment. After treatment, the expression of microRNA-92a, microRNA-217, microRNA-221, microRNA-222, microRNA-15a and microRNA-126 decreased significantly, and the expression of microRNA-200, microRNA-182, microRNA-429 and microRNA-497 increased significantly. After butylphthalide injection combined with acupuncture treatment, the expression of microRNA-92a, microRNA-217, microRNA-221 and microRNA-15a in serum decreased further, and the expression of microRNA-182 and microRNA-429 increased further. Acupuncture combined with butylphthalide injection can effectively improve motor function and cognitive function in patients with cerebral infarction, which may be related to the content of microRNA in serum.

**Key words:** microRNAs, acupuncture, butylphthalide, cerebral infarction, motor function, cognitive function

Cerebral infarction, also known as ischemic stroke, is mainly caused by occlusion of cerebral vessels or stenosis of cerebral vascular lumen caused by cerebral atherosclerosis and thrombosis. There is acute cerebral insufficiency, resulting in ischemic necrosis of local brain tissue, generally accompanied by nerve and motor function damage<sup>[1,2]</sup>. Therefore, the improvement of cerebral ischemia and cerebral ischemia is the focus of treatment. The recovery period of patients with cerebral infarction after stable condition is also a key stage to determine the recovery of motor function and cognitive function. Strengthening

the recovery treatment of patients with cerebral infarction is of great significance to improve the prognosis of patients<sup>[3,4]</sup>.

Butylphthalide is a new drug against cerebral ischemia injury. It can improve the ischemia and hypoxia of brain tissue, block the occurrence of brain injury and protect brain function<sup>[5]</sup>. It is also reported that early rehabilitation training has a significant effect on improving the prognosis of patients with cerebral infarction<sup>[6]</sup>. In recent years, Traditional Chinese Medicine (TCM) treatment technology has been gradually used in patient's treatment, and satisfactory results have been

---

\*Address for correspondence

E-mail: liuzike78@163.com

achieved. TCM acupuncture and moxibustion therapy inherits the essence of TCM. It is a TCM auxiliary treatment method often used in the rehabilitation period of patients with cerebral infarction. It regulates the operation of Qi and blood through acupuncture at relevant acupoint, so as to improve the phenomenon of blocked meridians<sup>[7,8]</sup>.

MicroRNA (miRNA) is a kind of small non coding RNA with a length of about 25 base pairs, which is encoded by endogenous genes and widely exists in eukaryotic cells. After specific binding with the target gene, it may regulate the role of the target gene by inducing the degradation of downstream targeted messenger Ribonucleic Acid (mRNA), inhibiting the initiation of protein translation or the extension of polypeptide chain, degrading new proteins and other ways<sup>[9,10]</sup>. At present, many studies have shown that miRNAs participate in a series of important physiological and pathological processes of organisms and are closely related to the occurrence and development of a variety of diseases such as tumors, hematopoietic system diseases, nervous system and cardiovascular diseases<sup>[11,12]</sup>.

This study explored the effect of butylphthalide injection combined with acupuncture on the recovery of motor function and cognitive function in patients with acute cerebral infarction. At the same time, the expression of multiple miRNAs in blood before and after treatment was detected to explore the expression changes of miRNAs in serum after butylphthalide injection combined with acupuncture treatment.

## MATERIALS AND METHODS

### General information:

106 patients with acute cerebral infarction hospitalized in the Department of Neurology of our hospital from July 2020 to November 2021 were selected. All patients were diagnosed as acute cerebral infarction by cranial Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and cerebrovascular imaging. The patients were randomly divided into control group and combined group, with 53 patients in each group. There were 31 male patients and 22 female patients in the control group. The average age was (63.97±8.06) y. There were 28 male and 25 female patients in the combined group. The average age

was (64.9±7.64) y. All patients had informed consent and signed the informed consent form.

### Inclusion criteria:

The patient met the diagnostic criteria of acute cerebral infarction. The patient had the first onset and the onset time was within 24 h. There is no contraindication to the drugs used in this study. The patient's condition was stable, and the patient and his family knew and agreed to join the study.

### Exclusion criteria:

Patients with recurrence of acute cerebral infarction; patients with severe communication and cognitive impairment and unable to communicate normally; patients with primary diseases such as severe cardiac insufficiency, active liver disease and renal insufficiency; patients with mental illness; patients with transient ischemic attack or reversible ischemic neurological deficit; patients with drug contraindications such as butylphthalide or severe bleeding tendency; patients who change their dressing without the permission of the doctor during the treatment; patients with a history of drug abuse and patients who do not agree to sign informed consent.

### Therapeutic methods:

The patients in the control group were treated with routine treatment such as anti-platelet aggregation, improving microcirculation, regulating lipid and stabilizing plaque, controlling blood pressure and blood glucose, maintaining water electrolyte balance and nutritional support, and were given butylphthalide sodium chloride injection (NBP Pharmaceutical Co., Ltd., Approval No: H20100041), 100 ml/time, 2 times/d and continuous medication for 2 w. The patients in the combined group were treated with acupuncture on the basis of the patients in the control group. Neiguan point, Sanyinjiao point, Shuigou point, Baihui Point, Sishencong point and Taichong point are selected on the acupoint (all on the affected side of the limb). If combined with limb dysfunction, other acupoint are added. Jiahegu point, Jianluo point and Waiguan point for upper limb dysfunction, and Yanglingquan point, Huantiao point, Kunlun point and Weizhong point for lower limb dysfunction, Jiadicang point and buccal car point with oblique mouth angle. During acupuncture treatment, 75 % ethanol was used to disinfect the skin of the acupuncture site, and Huatuo brand disposable

filiform needle (0.25×40.00) mm was used. During acupuncture treatment, the method of flat tonic and flat catharsis was used. After getting Qi, the needle was retained for 30 min, once a day, 5 times a week for 2 w.

#### Motor function assessment:

According to the Fugl Meyer Scale (FMA) of limb motor function, the motor function of the two groups before and after intervention was evaluated. It includes the evaluation of upper limb motor ability (66 points) and lower limb motor ability (34 points). The score is positively correlated with motor ability.

#### Cognitive function assessment:

Mini Mental State Examination (MMSE) scale was used to evaluate the cognitive function of the two groups before and after treatment. The scale includes visuospatial function, attention, naming, abstraction, delayed recall, orientation, language, MMSE scale and so on. The higher the score, the higher the level of cognitive function of the patients.

#### Quantitative Real-time polymerase chain reaction (qRT-PCR):

The peripheral blood of patients in each group was collected and serum samples were prepared. 10 ml of whole blood was collected with a blood collection tube containing coagulant. The whole blood was left standing at room temperature (25°) for 10 min, and 1900 g was centrifuged at 4° for 10 min. The upper liquid serum was absorbed into the sharp bottom Eppendorf (EP) tube, and 16 000 g was centrifuged at 4° for 10 min. the supernatant was absorbed into a new tube. The supernatant was the total RNA serum sample containing free miRNA. Then extract the total RNA containing free miRNA from the serum sample. See the instructions of miRNAs serum plasma kit of Qiagen company for specific operations. The method of detecting miRNAs expression was first reverse transcription and then real-time PCR. The reverse

transcription temperature was set at 42° for 60 min and 85° for 5 s. The temperature setting conditions of real-time PCR were 95° for 5 min, 95° for 5 s, 55° for 5 s, 70° for 3 s and 45 cycles. The sample dosage is operated according to the instructions, and the expression amount of miRNAs takes U6 as the internal reference.

#### Statistical analyses:

All data in this study were statistically analyzed by Statistical Package for the Social Sciences (SPSS) 25.0 software (SPSS, Chicago, Illinois, United States of America (USA)). All measurement data are expressed in the form of mean±variance, and t-test is used for statistical analysis. p<0.05 means the difference is statistically significant.

## RESULTS AND DISCUSSION

Compare the FMA scores of the two groups before and after treatment (Table 1). There was no significant difference in FMA score between the two groups before treatment. After treatment, the FMA scores of the two groups were significantly higher than those before treatment. Compared with the control group, the FMA score of the combined group was significantly higher after treatment. The above results suggest that acupuncture combined with butylphthalide injection has a significant effect on the improvement of motor function in patients with cerebral infarction.

The total score of MMSE before and after treatment was compared between the two groups (Table 2). There was no significant difference in the total score of MMSE between the two groups before treatment. After treatment, the total score of MMSE in the two groups was significantly higher than that before treatment. Compared with the control group, the total score of MMSE in the combined group was significantly higher after treatment. The above results suggest that acupuncture combined with butylphthalide injection has a significant effect on the improvement of cognitive function in patients with cerebral infarction.

**TABLE 1: COMPARISON OF FMA SCORES BETWEEN THE TWO GROUPS BEFORE AND AFTER TREATMENT**

Group	n	Before treatment	After treatment	t	p
Control	53	30.61±4.95	58.94±6.72	24.710	0.000
Joint	53	31.75±4.19	73.86±8.29	33.000	0.000
t		1.280	10.180		
p		0.204	0.000		

**TABLE 2: COMPARISON OF MMSE TOTAL SCORES BETWEEN THE TWO GROUPS BEFORE AND AFTER TREATMENT**

Group	n	Before treatment	After treatment	t	p
Control	53	14.65±1.97	19.88±2.09	13.26	0.000
Joint	53	14.06±2.08	24.76±2.17	25.91	0.000
t		1.499	11.790		
p		0.137	0.000		

The expressions of miR-15a, miR-92a, miR-126, miR-182, miR-200, miR-217, miR-221, miR-222, miR-429 and miR-497 in the serum of the two groups were detected before and after treatment (fig. 1). The results showed that there was no significant difference in the expression of miR-15a, miR-92a, miR-126, miR-182, miR-200, miR-217, miR-221, miR-222, miR-429 and miR-497 between the two groups before treatment. After treatment, the expression of miR-92a, miR-217, miR-221, miR-222, miR-15a and miR-126 in the serum of the two groups decreased significantly, and the expression of miR-200, miR-182, miR-429 and miR-497 increased significantly. Compared with the control group, the expression levels of miR-92a, miR-217, miR-221 and miR-15a in the serum of the combined group were significantly decreased, and the expression levels of miR-182 and miR-429 were significantly increased.

Cerebral infarction is a common and frequently occurring disease in neurology. It can be caused by a variety of reasons, such as insufficient blood supply of local brain tissue, ischemic and hypoxic necrosis of brain tissue, various symptoms of neurological deficit and severe limb dysfunction. It is mainly divided into cerebral thrombosis, cerebral embolism, lacunar infarction and other types. Among them, cerebral thrombosis is more common, accounting for 60 % of all cerebral infarction, it is a common type of cerebral infarction. The functional recovery in the acute stage after cerebral infarction is particularly important to avoid or reduce complications, shorten hospital stay and restore the ability of daily living<sup>[13,14]</sup>.

Butylphthalide is a new drug for the clinical treatment of cerebral infarction. It can intervene the pathophysiological process after cerebral ischemia and hypoxia through a variety of mechanisms, improve the blood flow supply in the cerebral ischemic area, strengthen the metabolism of brain cells and promote the recovery of neurological function<sup>[15-17]</sup>. In addition, butylphthalide can also

promote the establishment of collateral circulation in cerebral ischemic area, so as to reduce the scope of infarction and reduce the degree of brain edema<sup>[18]</sup>. Studies have confirmed that it plays an obvious role in regulating the energy metabolism of brain tissue and inhibiting the apoptosis of brain tissue cells, and the application of butylphthalide can reduce the level of inflammatory factors and has a strong protective effect on brain function<sup>[19]</sup>. The results of this study also showed that after the injection of butylphthalide, the motor ability and cognitive function of patients could be significantly improved.

According to the theory of TCM, cerebral infarction is classified as "stroke", and it is considered that the main etiology and pathogenesis of the disease are internal obstruction of blood stasis, poor blood circulation and blocked Qi mechanism, and the syndrome performance of patients in rehabilitation period is more prominent<sup>[20,21]</sup>. The uncontrolled diet leads to the injury of the spleen. Phlegm dampness is endogenous, which leads to depression and heat, and phlegm turbidity is not removed. Affected by emotional and dietary factors, it leads to the disturbance of wind and phlegm, which leads to the onset of disease. Phlegm turbidity can lead to the disorder of the movement of Qi and blood. If it is combined with the endogenous blood stasis and the obstruction of blood vessels in the acute stage, these will lead to the syndrome of phlegm stasis, therefore, phlegm stasis plays an important role in the occurrence and development of acute cerebral infarction<sup>[22]</sup>. TCM believes that acupuncture therapy has the therapeutic effects of removing blood stasis and dredging collaterals, supporting righteousness and eliminating evil, calming wind and resolving phlegm<sup>[23]</sup>. In this study, Baihui point, located at the midpoint of the head, is selected for treatment. This point belongs to the governor's pulse and plays the role of opening the orifices and waking up the mind. It can improve the blood supply of the brain and balance



the Qi and blood in the brain<sup>[24,25]</sup>. Acupuncture at Shuigou point and Sishencong point has a strong effect of opening the orifices and waking the mind. Sanyinjiao point has the effect of harmonizing Qi, blood and Yin channels, strengthening the function of kidney, spleen and lung, and increasing the function of dredging collaterals and removing blood stasis. Neiguan point and Taichong Point have the effects of soothing the liver, calming the liver, quenching wind and stopping spasm<sup>[26,27]</sup>. Therefore, the combined use has the effects of dredging collaterals and removing blood stasis, opening orifices and refreshing the brain. In addition, acupuncture at Shuigou point and Baihui point will promote nerve repair and improve cerebral blood supply. Acupuncture at Sanyinjiao can slow down hypercoagulability and improve microcirculation<sup>[28]</sup>. Other studies have shown that acupuncture can effectively alleviate the level of muscle tension and spasm in patients with cerebral infarction and promote motor coordination<sup>[29]</sup>. The results also showed that after using butylphthalide combined with acupuncture, the recovery of motor function and cognitive function was better. This shows that butylphthalide injection combined with acupuncture can significantly improve motor function and cognitive function in patients with

cerebral infarction.

At the same time, the expression and retrograde detection of miRNAs in serum of each patient before and after treatment was done. The results showed that after treatment, the expression of miR-92a, miR-217, miR-221, miR-222, miR-15a and miR-126 decreased significantly, and the expression of miR-200, miR-182, miR-429 and miR-497 increased significantly. After butylphthalide injection combined with acupuncture treatment, the expression of miR-92a, miR-217, miR-221 and miR-15a in serum decreased further, and the expression of miR-182 and miR-429 increased further. This suggests that butylphthalide injection combined with acupuncture may improve the motor and cognitive function of patients with cerebral infarction by affecting the expression and secretion of miRNAs.

In conclusion, this study uses butylphthalide injection combined with acupuncture to treat patients with cerebral infarction, which proves that the combination of traditional acupuncture and modern western medicine can promote the recovery of motor function and cognitive function of patients with cerebral infarction and improve the clinical curative effect.

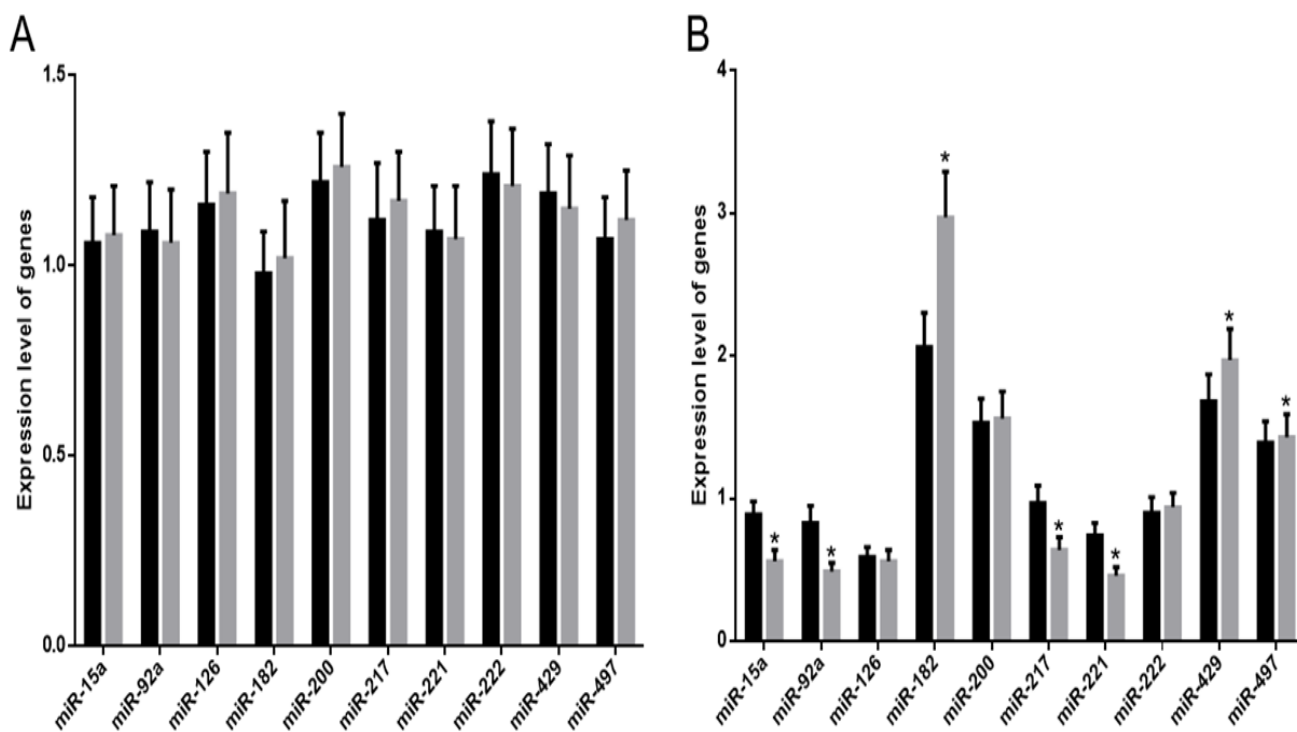


Fig. 1: The expression level of miRNAs in serum of patients with cerebral infarction before and after treatment. (A): The expression level of miRNAs in serum of the two groups before treatment and (B): The expression level of miRNAs in serum of the two groups after treatment  
Note: \* $p < 0.05$  compared with the control group, (■): Control group and (■): Joint group

**Conflict of interests:**

The authors declared no conflict of interests.

**REFERENCES**

- Zhao YJ, Nai Y, Ma QS, Song DJ, Ma YB, Zhang LH, *et al.* DI-3-n-butylphthalide protects the blood brain barrier of cerebral infarction by activating the Nrf-2/HO-1 signaling pathway in mice. *Eur Rev Med Pharmacol Sci* 2018;22(7):2109-18.
- Wang M, Feng Y, Yuan Y, Gui L, Wang J, Gao P, *et al.* Use of l-3-n-Butylphthalide within 24 h after intravenous thrombolysis for acute cerebral infarction. *Complement Ther Med* 2020;52:102442.
- Wang G, Ma D, Wang R. Effect of butylphthalide on serum CRP, PARK7, NT-3 and neurological function in patients with acute cerebral infarction. *Am J Transl Res* 2021;13(9):10388-95.
- Bu X, Xia W, Wang X, Lu S, Gao Y. Butylphthalide inhibits nerve cell apoptosis in cerebral infarction rats *via* the JNK/p38 MAPK signaling pathway. *Exp Ther Med* 2021;21(6):565.
- 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(1):361.
- Huang Y, Pan L, Wu T. Improvement of cerebral ischemia-reperfusion injury by l-3-n-butylphthalide through promoting angiogenesis. *Exp Brain Res* 2021;239(1):341-50.
- Liao W, Zhong Y, Cheng W, Dong LF. 3-N-butylphthalide inhibits neuronal apoptosis in rats with cerebral infarction *via* targeting P38/MAPK. *Eur Rev Med Pharmacol Sci* 2019;23(3):144-52.
- Qi FX, Hu Y, Wang S. Clinical observation of thrombolytic effect of alteplase combined with butylphthalide in patients with acute anterior circulation cerebral infarction. *Pak J Med Sci* 2021;37(4):1145-50.
- Bai J. Clinical efficacy and safety of urinary kallidinogenase combined with butylphthalide in the treatment of progressive cerebral infarction. *Am J Transl Res* 2021;13(12):13909-15.
- Zhang XL, Dong YT, Liu Y, Zhang Y, Li TT, Hu FY. Effects of dl-3-n-butylphthalide on serum lipoprotein-associated phospholipase A2 and hypersensitive C-reactive protein levels in acute cerebral infarction. *Brain Behav* 2019;9(12):e01469.
- Liu RZ, Fan CX, Zhang ZL, Zhao X, Sun Y, Liu HH, *et al.* Effects of DI-3-n-butylphthalide on cerebral ischemia infarction in rat model by mass spectrometry imaging. *Int J Mol Sci* 2017;18(11):2451.
- Zhang ZH, Zhang XC, Ni GX. Thrombolysis combined with acupuncture therapy for acute cerebral infarction: A meta-analysis of randomized controlled trials. *Zhen Ci Yan Jiu* 2021;46(5):431-8.
- Niu H, Zhang Z, Wang H, Wang H, Zhang J, Li C, *et al.* The impact of butylphthalide on the hypothalamus-pituitary-adrenal axis of patients suffering from cerebral infarction in the basal ganglia. *Electron Physician* 2016;8(1):1759.
- Li C, Chai A, Gao Y, Qi X, Zheng X. Combination of tetrandrine and 3-n-butylphthalide protects against cerebral ischemia-reperfusion injury *via* ATF2/TLR4 pathway. *Immunopharmacol Immunotoxicol* 2021;43(6):749-57.
- Xie K, Zhao S, Li X. Efficacy and mechanism of butylphthalide combined with atorvastatin calcium tablets in the diagnosis of cerebral infarction using Iodol/Fe<sub>3</sub>O<sub>4</sub> nanometric contrast agent. *J Nanosci Nanotechnol* 2020;20(12):7356-61.
- Song K, Zeng X, Xie X, Zhu R, Liang J, Chen G, *et al.* DI-3-n-butylphthalide attenuates brain injury caused by cortical infarction accompanied by cranial venous drainage disturbance. *Stroke Vasc Neurol* 2022;7(3):222-36.
- Wo X, Han J, Wang J, Wang X, Liu X, Wang Z. Sequential butylphthalide therapy combined with dual antiplatelet therapy in the treatment of acute cerebral infarction. *Pak J Med Sci* 2020;36(4):615.
- Han H, Li X, Jiang HN, Xu K, Wang Y. Effect of early acupuncture on cognitive function in patients with vascular dementia after cerebral infarction. *Zhongguo Zhen Jiu* 2021;41(9):979-83.
- Wei L, Zeng K, Gai J, Zhou F, Wei Z, Bao Q. Effect of acupuncture on neurovascular units after cerebral infarction in rats through PI3K/AKT signaling pathway. *Clin Hemorheol Microcirc* 2020;75(4):387-97.
- Wang Y, Xing J, Li Y, Zhang R. Effect and safety of acupuncture on cerebrovascular reserve in patients with acute cerebral infarction: A protocol for systematic review and meta-analysis. *Medicine* 2021;100(28):e26636.
- Yan J, Dong Y, Niu L, Cai J, Jiang L, Wang C, *et al.* Clinical effect of Chinese herbal medicine for removing blood stasis combined with acupuncture on sequelae of cerebral infarction. *Am J Transl Res* 2021;13(9):10843.
- Wang J, Ran C, Pan P, Wang Y, Zhao Y. Rehabilitation training combined acupuncture for limb hemiplegia caused by cerebral infarction: A protocol for a systematic review of randomized controlled trial. *Med* 2021;100(1):e23474.
- Qin C, Zhou P, Wang L, Mamtilahun M, Li W, Zhang Z, *et al.* DI-3-N-butylphthalide attenuates ischemic reperfusion injury by improving the function of cerebral artery and circulation. *J Cereb Blood Flow Metab* 2019;39(10):2011-21.
- Zhu BL, Xie CL, Hu NN, Zhu XB, Liu CF. Inhibiting of GRASP65 phosphorylation by DL-3-N-butylphthalide protects against cerebral ischemia-reperfusion injury *via* ERK signaling. *Behav Neurol* 2018;2018:5701719.
- Zhang C, Zang Y, Song Q, Zhao W, Li H, Hu L, *et al.* Effects of butylphthalide injection on treatment of transient ischemic attack as shown by diffusion-weighted magnetic resonance imaging abnormality. *Int J Neurosci* 2020;130(5):454-60.
- Yin J, Chang H, Wang D, Li H, Yin A. Fuzzy C-means clustering algorithm-based magnetic resonance imaging image segmentation for analyzing the effect of edaravone on the vascular endothelial function in patients with acute cerebral infarction. *Contrast Media Mol Imaging* 2021;2021:4080305.
- Yan RY, Wang SJ, Yao GT, Liu ZG, Xiao N. The protective effect and its mechanism of 3-n-butylphthalide pretreatment on cerebral ischemia reperfusion injury in rats. *Eur Rev Med Pharmacol Sci* 2017;21(22).
- Chen Y, Huang W, Li Z, Duan Y, Liang Z, Zhou H, *et al.* The effect of acupuncture on the expression of inflammatory factors TNF- $\alpha$ , IL-6, IL-1 and CRP in cerebral infarction: A

protocol of systematic review and meta-analysis. *Medicine* 2019;98(24):e15408.

29. Kim JH, Han JY, Song MK, Park GC, Lee JS. Synergistic effects of scalp acupuncture and repetitive transcranial magnetic stimulation on cerebral infarction: A randomized controlled pilot trial. *Brain Sci* 2020;10(2):87.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms

**This article was originally published in a special issue, "Exploring the Role of Biomedicine in Pharmaceutical Sciences" Indian J Pharm Sci 2024;86(1) Spl Issue "262-268"**