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Moxibustion for cognitive impairment: a systematic review and meta-analysis of animal studies

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ABSTRACT

Background: Cognitive impairment is an age-dependent chronic disorder that exponentially worsens with age; however, its treatment is mostly symptomatic. Moxibustion is widely accepted in East Asia as a treatment for cognitive impairment. This systematic review aimed to verify the efficacy and underlying mechanism of moxibustion in treating cognitive impairment.
Methods: Sixteen trials involving 324 animals obtained from MEDLINE (PubMed), EMBASE, the Cochrane library, the Chinese National Knowledge Infrastructure, Wan-Fang, Cqvip, the Korean Studies Information Service System, and the Oriental Medicine Advanced Searching Integrated System met the inclusion criteria. We extracted the results of behavioral tests and immunohistochemical biomarkers from the included articles and evaluated the risk of bias and reporting quality.
Results: The moxibustion group showed significantly decreased escape latency, increased crossing times, and prolonged dwelling times in the Morris water maze test. There was a significantly enhanced latency period and reduced error time in the step-down test and nerve behavior score. The effects of moxibustion were found to be mediated by suppression of oxidative stress and apoptosis, modulation of inflammation and Aβ genesis activation of vascular endothelial growth factor, and adjustment of metabolites in the tricarboxylic acid cycle and fatty acid metabolism.
Conclusion: Our results demonstrated the therapeutic efficacy of moxibustion on cognitive impairment and suggested the putative mechanism. However, considering the small number of included studies, high bias risk, low reporting quality, and the limitations of animal experimentation, our results need to be confirmed by more detailed studies.

1. Introduction

Cognitive impairment is an age-dependent chronic disorder[1]; globally, the number of patients is expected to reach 82 million in 2030 and 152 million in 2050.[2] Alzheimer disease (AD) is the most common type of cognitive impairment,[3] accounting for 60–80% of all cases,[2,4] which is characterized by the accumulation of Aβ peptides, senile plaques, and intracellular neurofibrillary tangles (NFT), related to neuronal damage and premature neuronal death.[4] Vascular dementia (VD) is the second most common type of cognitive impairment, which is driven by complex factors reducing cerebral blood flow and causing oxidative stress, resulting in cerebral ischemia.[5]

Despite the growing need for proper management of cognitive impairment in aging societies, the available therapeutic options are still mostly symptomatic, providing short-term benefit for specific condition, with a risk of adverse drug reactions (ADRs).[6]

Moxibustion is a widely used therapy in Eastern Asia for more than 2500 years.[7,8] Moxibustion entails stimulating acupoints on the body by burning herb leaves directly (attaching moxa cones to the skin) or indirectly (interposing a substance such as ginger between the moxa cones and the skin) to transmit heat stimulation[8,9] and induce pharmacological action via herbal components.[10,11]

In addition to its wide range of use for pain relief[12,13] and inflammation control,[14,15] moxibustion has been recognized as a suitable treatment for cognitive impairment, with several recent studies indicating its efficacy in patients with dementia.[16,17]

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Acupuncture, another representative traditional Chinese medicine treatment, has been reported to be effective in cognitive enhancement, with supporting evidence from several systematic reviews (SRs),[18,19,20] suggesting that its potential mechanism involves suppression of oxidative stress and neuroinflammation and modulation of glucose metabolism[5] and neuronal signaling pathways.[21]

However, compared with acupuncture studies, the therapeutic efficacy of moxibustion for cognitive impairment has not been fully validated. Although a previous study has reported the action of moxibustion in preventing cognitive impairment,[22] information on the overall therapeutic effect of moxibustion was limited, as it was focused on prevention. This SR aimed to evaluate the efficacy of moxibustion in treating cognitive impairment through a meta-analysis of animal studies. We also examined the underlying mechanisms and the quality of the supporting evidence.

2. Methods

2.1. Study search and selection

We used search terms consisting of variants of "cognitive impairment" for the target disease, "moxibustion" for the intervention, and "animal study." The following databases were searched: MEDLINE (PubMed), EMBASE, and the Cochrane library (English language); the Chinese National Knowledge Infrastructure, Wan-Fang, and Cqvip Database (Chinese language); the Korean Studies Information Service System and Oriental Medicine Advanced Searching Integrated System (Korean language). We searched studies published from the time of inception of each database to January 2019.

2.2. Inclusion and exclusion criteria

We included animal studies that conducted randomized controlled trials on moxibustion as an intervention for cognitive disorders. We did not discriminate between different types of cognitive impairment including AD and VD. The search terms we used for describing cognitive impairment are provided in Supplementary material. All rodent models developed for any type of cognitive impairment were included.

Although we included all types of moxibustion, we excluded studies that used combined treatments. Furthermore, in the control group, we only included studies that used cognitively impaired animals without any interventions. Regarding outcome measurements, we included results from behavioral tests, immunohistochemistry, and electron microscopic analyses.

Two reviewers (SMA and SC) independently selected and evaluated the studies and subsequently discussed for confirmation. Conflicts were resolved by consulting a third reviewer (UMJ) to reach a consensus. We used the eligible studies to extract data in a standardized form suitable for animal study design as follows: study design, research institute, methodological details, procedure compatibility (performing of randomization and blinding), and therapeutic characteristics of moxibustion. Regarding measurement outcomes, we defined the primary outcomes as behavioral test results and the secondary outcomes as immunohistochemical results. For studies where data was only graphically presented, we attempted to contact the corresponding author; if unsuccessful, we extracted the data by scaling the graph using the GetData graph digitizer version 2.26.0.20 (copyright 2001–2013; S. Fedorov). Details of the included studies are shown in Table 1.

2.3. Study assessment and analysis

We evaluated the overall potential bias in the included studies based on the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES)

Table 1
Characteristics of the included studies in animal model and treatment.

Study	Animal model						Treatment			
	Species	Age (months)	Weight (g)	Sample size		Target Disease	Acupoints	Moxa diameter (mm)	Duration (min)	Treatment period
				Moxa (M, F)	Control (M, F)					
Du [26]	Wistar rat	12	500 ± 20	(10,0)	(10,0)	AD	GV20, BL23	6	15	14 times for 14 days
Liu [33]	ApoE(−/−) mice	2	27 ~ 29	(11,0)	(11,0)	AD	CV8	20~30	20	72 times for 78 days
Wang [27]	SD rat	15	350 ~450	(10,0)	(10,0)	AD	GV20, BL23, ST36	NR	5	18 times for 21 days
Wang [28]	SD rat	15	350 ~450	(10,0)	(10,0)	AD	GV20, BL23, ST36	8	5	18 times for 21 days
Wang [29]	SD rat	15	350 ~450	(10,0)	(10,0)	AD	GV20, BL23, ST36	NR	5	18 times for 21 days
Jiang [31]	SD rat	2	300 ± 30	(11,0)	(10,0)	AD	GV20, GV4, GV1, CV4	10	3 or 7*	10 times for 30 days
Wang [34]	Wistar rat	NR	300 ± 30	(12,0)	(12,0)	VD	GV20, GV24, GV14	NR	20	30 times for 31 days
Wang [37]	Wistar rat	10	300 ± 30	(12,0)	(12,0)	VD	GV20, GV24, GV14	2~3	20	30 times for 31 days
Zhu [30]	SD rat	NR	NR	(12,0)	(12,0)	AD	GV20, GV16, GV14	20	20	15 times for 15 days
Wang [35]	SD rat	NR	250 ± 10	(7,0)	(7,0)	VD	GV20, GV24, GV14	5	20	30 times for 30 days
Weilan [38]	Wistar rat	NR	220 ± 20	(7,0)	(7,0)	VD	GV20, GV16, ST36, GB20	NR	3 Zhuang	24 times for 27 days
Luo [40]	Wistar rat	NR	280 ± 20	(10,0)	(10,0)	VD	CV6, CV12, CV17, SP10, TE5, ST36	5	3 or 4 Zhuang	24 times for 27 days
Zhang [36]	Wistar rat	10	250 ± 50	(8,0)	(8,0)	VD	GV20, GV24, GV14	15	20	30 times for 31 days
Xiao [7]	SD rat	2	350 ~400	(10,0)	(10,0)	AD	KI6, TE5, GB41, PC6, SP4, SI3, BL62, LU7	NR	9 Zhuang	35 times for 35 days
Yu [32]	APP/PS1mice	6	NR	(12,0)	(12,0)	AD	CV4	20	15	48 times for 55 days
Zhu [39]	SD rat	NR	200 ~250	(10,0)	(10,0)	VD	GV14, GV4, CV4	NR	15	24 times for 27 days

Abbreviations: SDS, prague-Dawley; ApoE−/−, apolipoprotein E-deficient; APP/PS1, Amyloid precursor protein/presenilin-1 transgenic; M, male; F, female; AD, Alzheimer's disease; DB, database; VD, vascular dementia; NR, not reported; NA, not applicable.
* Jiang[31] treated all participants using moxibustion for 3 min except GV20 which received moxibustion for 7 min.

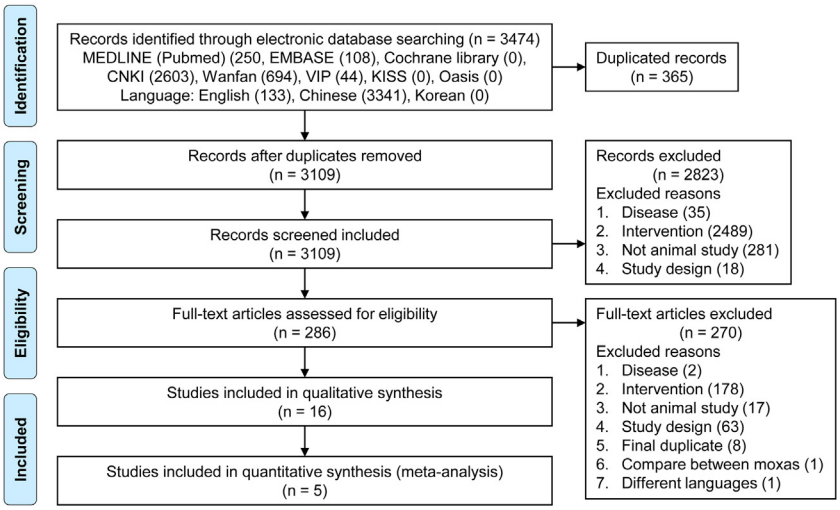


Fig. 1. PRISMA flow diagram for selecting related studies.

study quality checklist,[23] as suggested by CAMARADES[24] for reporting animal data from experimental studies. The reporting quality of each study was evaluated according to the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines checklist.[25]

In the behavioral test, decreased escape latency in the Morris water maze (MWM) test suggests restored cognitive function, which is indicated by faster learning, maintaining a better score on average, and achieving a superior final score; meanwhile, increased cross and dwelling times from the target probe test suggest prolonged memory. Assessment of the step-down test results and neuronal behavior scores indicated a beneficial effect in extending the latency period and shortening error times.

The results of behavioral tests obtained by means and standard errors were synthesized, and meta-analyses were conducted using a random effect model, the RevMan version 5.3 (released on June 13, 2014, Cochrane Collaboration).

3. Results

3.1. Characteristics of included studies

3.1.1. Study screening

Based on our electronic search criteria, we retrieved 3474 articles. After removing duplicate studies, the titles and abstracts of 3109 studies were screened and 296 articles were selected for in-depth screening of the full text. Finally, 16 original articles met our inclusion criteria, of which five could be quantitatively synthesized. Among the 16 articles, two were dissertations not published in peer-review journals. A diagram of the study selection process is shown in Fig. 1.

3.1.2. Fundamental study characteristics: animals

The 16 original research studies included in our analysis used a total of 324 rats and mice (age: 2–15 months; weight: 200–520 g [rats], 27–29 g [mice]). Nine studies established an AD model; among them, four studies injected Aβ (Aβ1–42[26] or Aβ25–35[27–29]), three studies injected chemical toxin (D-galactose[7,30] or streptozotocin[31]) into the hippocampus, and two studies established transgenic mice (amyloid precursor protein [APP]/presenilin 1 [PS1]), double-transgenic mice,[32] or apolipoprotein E-deficient (ApoE−/−) mice.[33] Seven studies established VD models by performing four-vessel occlusion,[34–36] bilateral carotid artery occlusion,[37–39] or autologous blood injection.[40] The characteristics of the included studies are shown in Table 1.

3.1.3. Fundamental study characteristics: moxibustion

The most frequently used acupoint was GV20 (11 studies), followed by GV14 (6 studies), ST36 (5 studies), then GV24 and BL23 (4 studies; Supplementary material). The extra acupoints selected for treatment are described in Supplementary material. Indirect moxibustion was used in most of the studies, except for two studies where direct moxibustion was performed.[38,40] Among those that used indirect moxibustion, eight burned the moxibustion about 2–3 cm above the surface of the acupoints, four placed a moxibustion cone on the skin and burned it, and two stimulated the acupoints by dropping ashes of thread soaked in herb medicine on the acupoints. The total treatment period ranged from 2 to 11 weeks, and the most frequently set time was 4 weeks (9 studies).

3.2. Quality assessment of the included studies

3.2.1. Risk of bias

Based on the checklist for evaluating the risk of bias, on average about 5.3 criteria were satisfied by the included studies (Table 2). All studies reported the implementation of randomization and possible conflicts of interest. However, no study reported the blinding process of allocation and only one study performed the blinded assessment of outcome.

3.2.2. Reporting quality

Regarding the ARRIVE guidelines (Table 3), all studies fully presented four items, including the title, objectives, sample size per group, and funding. Regarding the experimental procedure, although most studies detailed “how it was conducted,” only four studies mentioned “why it was conducted.” Although all studies reported the sample size, the method and reference of sample calculation were not reported, and the assessment regarding the statistical approach was not properly disclosed.

3.2.3. Data acquisition for analysis

Regarding the analysis of the behavioral test results, we excluded studies that reported duplicated data[29] or omitted detailed values[33] from the outcome analysis. Furthermore, studies reporting graph-shaped results[33,35,41,42] were scaled by the GetData graph digitizer program, and the means and standard deviations were extracted.

Table 2
Risk of bias in the included studies evaluated by the CAMARADES' study quality checklist.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	Total count of Y
Du [26]	Y	Y	Y	N	N	Nn	Y	N	Y	Y	6
Liu [33]	Y	Y	Y	N	Y	Nn	Y	N	N	Y	6
Wang [27]	Y	Y	Y	N	N	Nn	Y	N	N	Y	5
Wang [28]	Y	Y	Y	N	N	Nk	Y	N	Y	Y	6
Wang [29]	Y	Y	Y	N	N	Nk	Y	N	Y	Y	6
Jiang[31]	Y	N	Y	N	N	Nk	Y	N	N	Y	4
Wang [34]	Y	N	Y	N	N	Nk	Y	N	N	Y	4
Wang [37]	Y	Y	Y	N	N	Nk	Y	N	Y	Y	6
Zhu [30]	Y	Y	Y	N	N	Nn	Y	N	N	Y	6
Wang [35]	Y	Y	Y	N	N	Nn	Y	N	Y	Y	6
Weilan[38]	Y	Y	Y	N	N	Nk	Y	N	Y	Y	6
Luo [40]	Y	Y	Y	N	N	Nk	Y	N	N	Y	5
Zhang [36]	Y	Y	Y	N	N	Nk	Y	N	N	Y	5
Xiao [7]	Y	Y	Y	N	N	Nn	Y	N	N	Y	5
Yu [32]	N	Y	Y	N	N	Nn	Y	N	N	Y	4
Zhu [39]	N	Y	Y	N	N	Nk	Y	N	Y	Y	5
Total count of Y	14	14	16	0	1	0	16	0	7	16	

(1) Publication in peer-reviewed journal (2) Statement of control of temperature (3) Randomization of treatment or control (4) Allocation concealment (5) Blinded assessment of outcome (6) Avoidance of anesthetics with marked intrinsic properties (7) Use of a suitable animal model (8) Sample size calculation (9) Statement of compliance with regulatory requirements (10) Statement regarding possible conflict of interest.
Y, Yes; N, No; Nn, Not necessary; Nk, Not known.

3.3. Outcome analysis of the included studies

3.3.1. Results of primary outcome: behavioral experiments

Among the studies that performed behavioral tests, those with duplicated[29] and insufficient data[33] were excluded, whereas results presented in the form of graphs[33,35,41,42] were converted to numerical values. Consequently, we obtained results from 11 behavioral tests and finally included five studies that performed the MWM test and two that performed the step-down and nerve behavioral tests for analysis.

3.2.2. Morris water maze test

Among the nine studies that performed MWM tests, five[26,30,31,36,40] that reported the individual results for MWM tests conducted for 4 days were included in the meta-analysis. Regarding escape latencies, the moxibustion group showed a significantly decreased escape latency compared to the control group after analysis of results measured from days 1 to 4 (Fig. 2). Additionally, from the 1st to the 3rd day, there was a decreasing tendency of the SMD of escape time (1st day: SMD: −1.97; 95% CI: −3.54, −0.40; $p = 0.01$, $I^2 = 90\%$; 2nd day: SMD: −3.19; 95% CI: −5.32, −1.06; $p < 0.001$, $I^2 = 93\%$; 3rd day: SMD: −4.51; 95% CI: −7.07, −1.96; $p < 0.001$, $I^2 = 93\%$). The greatest difference in the SMD between the moxibustion and control group was observed on the 3rd day.

Regarding platform crossing times, the moxibustion group presented significantly increased crossing times compared to the control group (SMD: 4.19; 95% CI: 1.98, 6.40; $p < 0.001$; Fig. 3A). The dwelling time was reported in four different formats as follows: time spent in the quadrant,[43,44,45] percentage of time dwelling,[30,33,41,42] percentage of total distance traveled within the quadrant,[46] and picture tracking of the animal's motion.[27,31] Meta-analysis indicated a significantly increased dwelling time in the quadrant in the moxibustion group compared with that in the control group (SMD: 2.49; 95% CI: −0.10, 5.07; $p < 0.001$); however, the heterogeneity remained high ($I^2 = 93\%$). Three studies,[27,31,46] albeit not included in the meta-analysis, showed significantly decreased distance within the quadrant where animals in the moxibustion group traveled.

3.2.3. Step-down test

Among the three studies that performed step-down tests, two studies[34,37] reporting same methods were meta-analyzed and showed significantly extended latent periods (SMD: 2.93; 95% CI: 2.03, 3.83; $p < 0.001$; Fig. 3B (A)) and shortened error times (SMD: −2.50; 95% CI: −3.70, −1.31; $p < 0.001$; Fig. 3B (B)) in the moxibustion group.

In another study,[33] the moxibustion group showed significantly prolonged latency and decreased error counts in both training and retention stages.

3.2.4. Nerve behavior score

Two of the studies[34,37] that conducted step-down tests also determined nerve behavior scores. The results were scored by a five-point scale in reference to a previous study[47] Consequently, we found significantly decreased nerve behavior scores in the moxibustion group compared with the control group (SMD: −2.09; 95% CI: −2.82, −1.36; $p < 0.001$; Fig. 3C).

3.2.5. Results of the secondary outcome: putative immunohistochemical biomarkers

Fourteen of the included studies investigated various immunohistochemical biomarkers for pathogenic characteristics (Table 4 and Fig. 4).

Liu et al[33] reported decreased levels of glial fibrillary acidic protein (GFAP), whereas three studies[30,31,39] verified attenuated inflammatory damage after moxibustion treatment. Three studies[7,27,38] demonstrated apoptosis-related factors that were controlled after moxibustion treatment; they also reported the upregulation of B-cell lymphoma 2 (Bcl-2) and downregulation of Bcl-2–associated X protein (Bax). The morphological changes of neuronal cells in the control group were compared with those of the moxibustion group,[29,30] and recovery of morphological characteristics of neuronal cells was observed in the moxibustion group. Four studies[28,30,31,33,41] investigated Aβ genesis and found it to be significantly restrained in the moxibustion group, with Zhu et al[30] reporting decreased levels of PS1 and β-site APP cleaving enzyme 1 (BACE-1) in the moxibustion group, indicating the efficacy of moxibustion in regulating Aβ genesis.

Two studies[34,37] found that moxibustion improved VEGF levels and related factors. Furthermore, Zhang et al[36] postulated that proliferation and migration of vascular endothelial cells occurred in the moxibustion group. Yu[32] also investigated attenuated metabolites of the TCA cycle and fatty acid metabolism after moxibustion.

Table 3
Reporting quality assessment of the treatment studies based upon the ARRIVE guidelines.

Study	ARRIVE Guideline																																				
	Introduction								Methods																		Results						Discussion						
	Title	Abstract	Background		Objectives	Ethical statement	Study design			Experimental procedure				Experimental animals		Housing/husbandry			Sample size			Allocating animals to experimental groups		Experimental outcomes	Statistical methods			Baseline data	Numbers analyzed		Outcomes and estimation	Adverse events		Interpretation/ scientific implications			Generalizability / Translation	Funding
			a	b			a	b	c	a	b	c	d	a	b	a	b	c	a	b	c	a	b		a	b	c		a	b		a	b	a	b	c		
Du [26]	F	P	F	F	F	F	F	P	P	F	P	P	N	F	P	F	F	N	F	N	NA	P	N	F	P	F	N	P	F	NA	F	N	N	F	F	P	F	F
Liu [33]	F	P	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	N	F	N	NA	F	N	F	F	F	N	P	F	P	P	N	N	F	F	N	F	F
Wang [27]	F	P	P	P	F	N	F	F	P	F	P	F	N	F	F	P	P	N	F	N	NA	P	N	F	P	F	N	F	F	NA	F	N	N	F	N	N	P	F
Wang [28]	F	P	P	P	F	N	F	F	P	F	F	F	N	F	F	N	N	N	F	N	NA	P	N	F	P	F	N	P	F	NA	F	N	N	F	N	N	P	F
Wang [29]	F	P	P	P	F	F	F	P	P	F	F	F	F	F	F	P	P	N	F	N	NA	P	N	F	F	F	N	F	F	F	F	N	N	F	N	N	P	F
Jiang[31]	F	P	P	P	F	F	F	F	P	F	F	F	F	F	F	F	P	P	F	N	NA	P	N	F	F	F	N	N	F	F	F	N	N	F	N	N	P	F
Wang [34]	F	P	F	P	F	N	F	P	F	F	F	F	P	F	F	F	P	P	F	N	NA	F	N	F	F	F	N	N	F	F	F	N	N	P	N	N	P	F
Wang [37]	F	P	F	P	F	N	F	F	F	F	F	F	N	F	F	F	P	P	F	N	NA	F	N	F	F	F	N	N	F	F	F	N	N	P	N	N	N	F
Zhu [30]	F	P	F	P	F	F	F	P	F	F	F	F	N	P	F	P	P	N	F	N	NA	F	N	F	F	F	N	P	F	F	F	N	N	F	N	N	N	F
Wang [35]	F	P	F	P	F	N	F	P	F	F	F	F	N	F	F	F	N	N	F	F	NA	F	N	F	NA	F	N	P	F	F	F	N	N	F	N	N	N	F
Weilan[38]	F	P	F	P	F	F	F	P	F	F	F	F	N	F	F	F	F	P	F	N	NA	F	N	F	F	F	N	P	F	F	F	N	N	F	N	N	P	F
Luo [40]	F	P	F	P	F	N	F	F	F	F	F	F	N	F	F	F	F	P	F	N	NA	F	N	F	F	F	N	F	F	F	F	N	N	F	P	N	P	F
Zhang [36]	F	P	F	P	F	N	F	F	F	F	F	F	N	F	F	F	P	P	F	N	NA	F	N	F	F	F	N	F	F	F	F	N	N	F	P	N	P	F
Xiao [7]	F	P	F	P	F	N	F	P	F	F	F	F	N	F	F	P	F	P	F	N	NA	F	N	F	F	F	N	P	F	F	F	N	N	F	F	N	P	F
Yu [32]	F	F	F	P	F	N	F	F	F	F	F	F	N	F	F	P	F	N	F	N	NA	F	N	F	F	F	N	P	F	NA	F	N	N	F	F	N	P	F
Zhu [39]	F	F	F	P	F	F	F	F	F	F	F	F	N	F	F	P	F	N	F	N	NA	F	N	F	F	F	F	F	F	F	F	N	N	F	F	N	P	F

Abbreviations: ARRIVE, Animal Research: Reporting In Vivo Experiments; F, fully reported; P, partially reported; N, not reported; NA, not applicable.

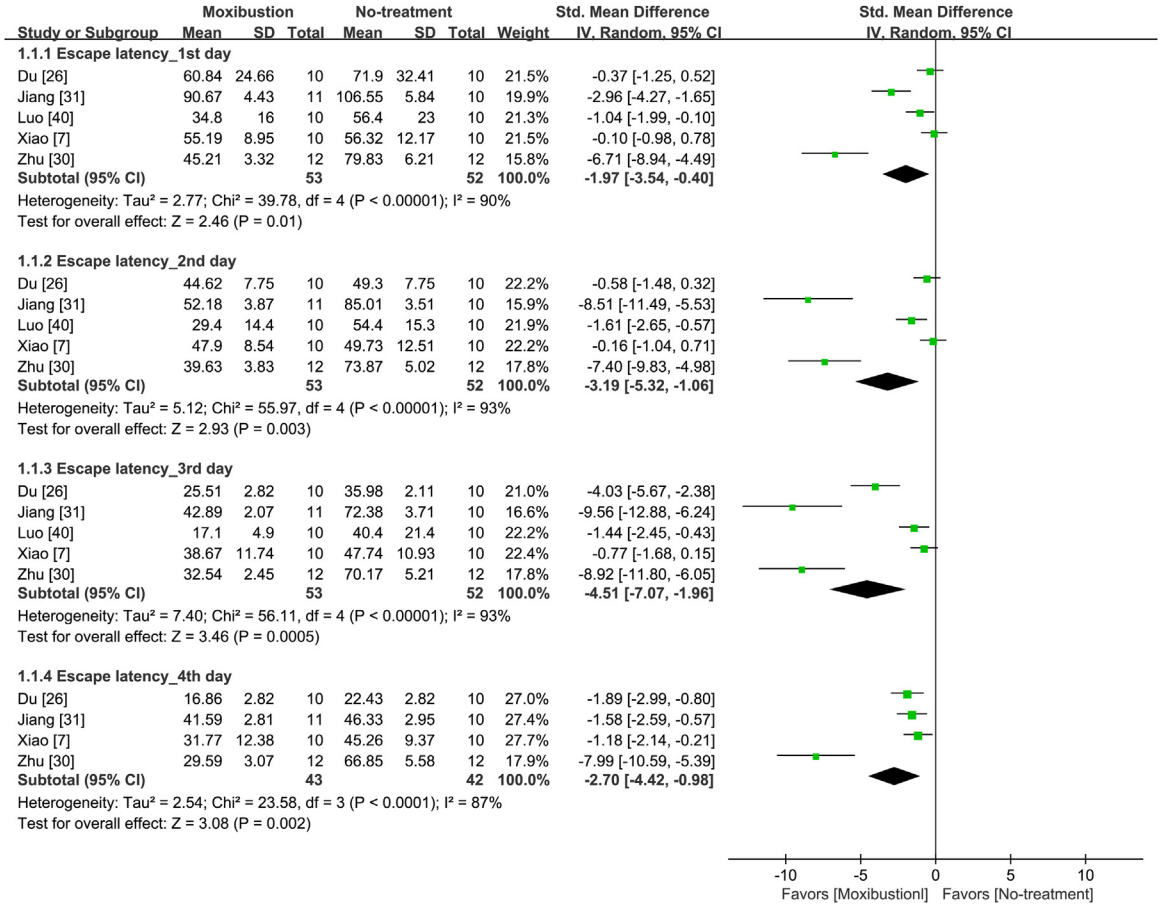


Fig. 2. Forest plot of escape latency in the Morris water maze test from the 1st to 4th day.

Table 4
Outcomes evaluated in the included studies.

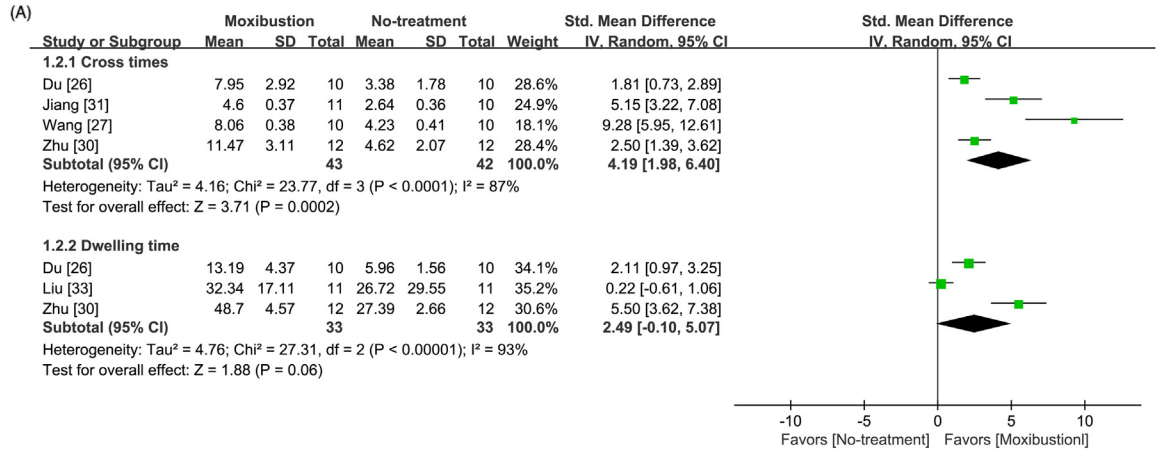
Study	Outcome Behavioral test	Signal pathway
Du [26]	1. Morris water maze a) Escape latency b) Crossing times c) Dwelling time	Apoptosis rates ↓
Liu [33]	1. Morris water maze c) Dwelling time	GFAP ↓, Aβ ↓
Wang [27]	1. Morris water maze a) Escape latency b) Crossing times	Bcl-2 ↑, Bax ↓, Caspase-3 ↓
Wang [28]	None	Aβ ↓
Wang [29]	Duplicated with Wang [27]	Morphologic change
Jiang [31]	1. Morris water maze b) Crossing times	IL-1β ↓, IL-2 ↑, Aβ ↓
Wang [34]	2. Step-down test a) Latent period b) Error times 3. Nerve behavior score	VEGF ↑, flt-1 ↑, flk-1mRNA ↑
Wang [37]	2. Step-down test a) Latent period b) Error times 3. Nerve behavior score	VEGF ↑, flt-1 ↑, bFGF ↑, bFGF-r ↑
Zhu [30]	1. Morris water maze a) Escape latency b) Crossing times c) Dwelling time	PS1 ↓, BACE-1 ↓, serum IL-6 ↓, Aβ ↓
Wang [35]	None	morphologic change
Weilan [38]	NA	Bax ↓, Bcl-2 ↑, C-fos ↓
Luo [40]	1. Morris water maze a) Escape latency b) Crossing times	NA
Zhang [36]	NA	Vascular endothelial cell proliferation↑, migration↑
Xiao [7]	1. Morris water maze a) Escape latency	Bax ↓, Bcl-2 ↑, SOD2 ↑, MDA ↓, GSH-Px ↑ ChAT ↑, AChE ↓
Yu [32]	NA	Metabolites of TCA ↑ Metabolites of fatty acid ↓ Mono / polyunsaturated fatty acids ↑
Zhu [39]	1. Morris water maze a) Escape latency	NF-κB (NF-κBp65, NFκBp50) ↓, TNF-α ↓, IL-1β ↓, morphologic change

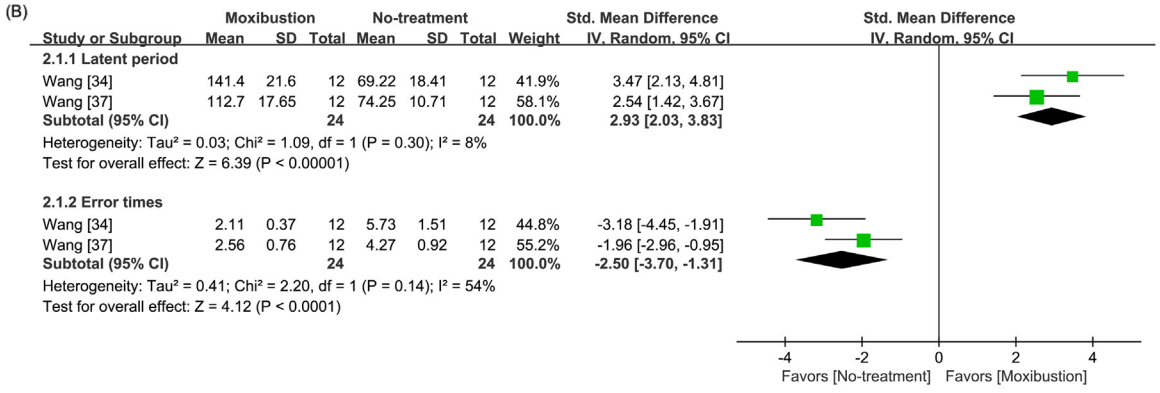
Abbreviations: TCA, tricarboxylic acid; FA, fatty acid; P-p38 MAPK, phospho-p38 mitogen-activated protein kinase; Aβ, β-amyloid; GFAP, glial fibrillary acidic protein; IL, interleukin; VEGF, vascular endothelial growth factor; Flt-1, fms-like tyrosine kinase 1; bFGF, basic fibroblast growth factor; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; SOD, superoxide dismutase; MDA, malondialdehyde; BACE-1, β-site APP cleaving enzyme 1; PS1, presenilin-1; APP, Amyloid precursor protein; GSH-Px, glutathione peroxidase; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; TNF-α, tumor necrosis factor-α; NF- kB, nuclear factor-kappa.

4. Discussion

4.1. Summary of the main findings

The outcomes of this SR verified the efficacy of moxibustion in the treatment of cognitive impairment. Moxibustion treatment significantly improved the results of the behavioral test, the primary outcomes; it also showed efficacy against neurodegenerative disorders as shown through the outcomes of the immunohistochemical biomarkers, the secondary outcomes. These effects include attenuated acetylcholine (ACh) deficit and mitochondrial oxidative stress, attenuated inflammation and APP secretion,





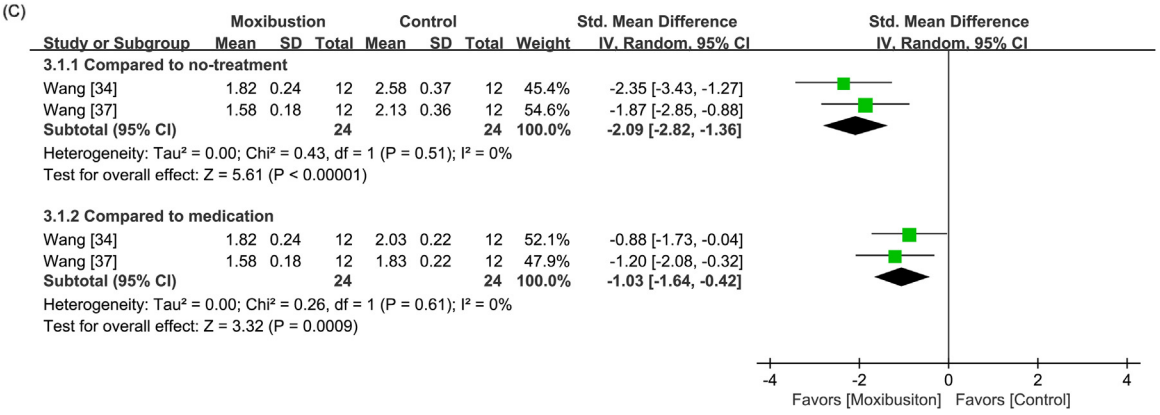


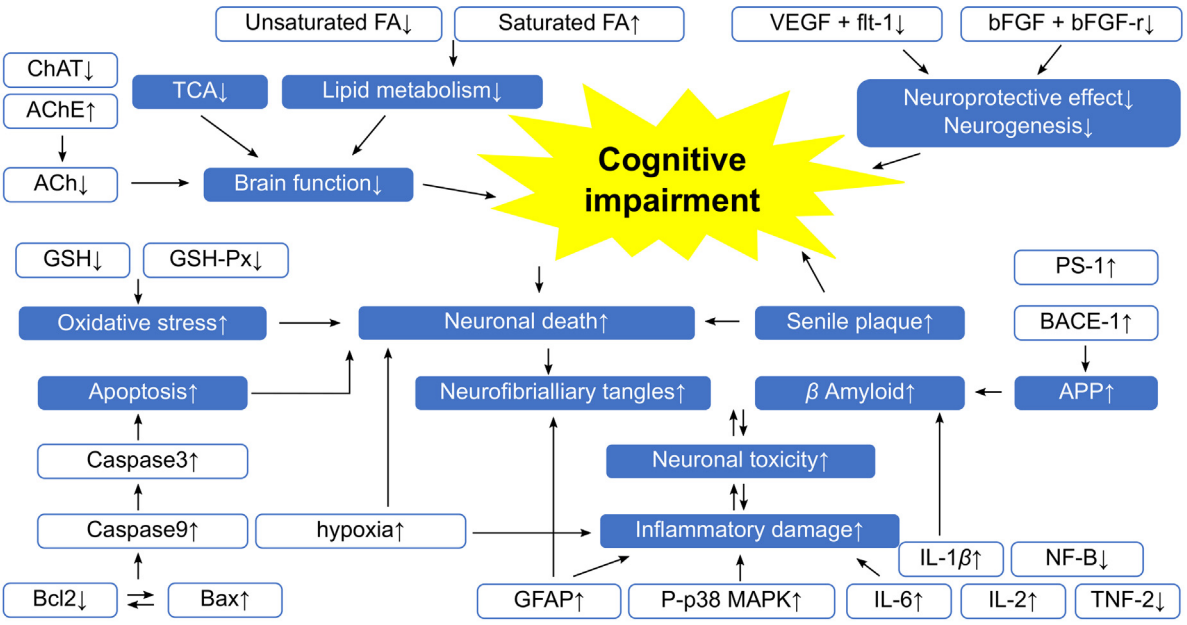
Fig. 3. Forest plot of (A) cross times and dwelling time in the Morris water maze test (B) latency period and error times in the step-down test. (C) nerve behavior score.

enhanced vascular endothelial growth factor (VEGF) activity and proliferation of vascular endothelial cells, and modulation of metabolites of the tricarboxylic acid (TCA) cycle and fatty acid metabolism.

4.2. Overall completeness and applicability of the evidence

4.2.1. Suppressing ACh deficits and mitochondrial oxidative stress

The accumulation of Aβ1–42 has been reported to ACh deficit[48] related oxidative stress,[49] which leads to metabolic malfunctions



Abbreviations: TCA, tricarboxylic acid; FA, fatty acid; P-38 MAPK, phospho-p38 mitogen-activated protein kinase; Aβ, β-amyloid; IL, interleukin; GFAP, glial fibrillary acidic protein; VEGF, vascular endothelial growth factor; Flt-1, fms-like tyrosine kinase 1; bFGF, basic fibroblasst growth factor; Bax, Bcl-2 associated X protein; BACE-1, β-site APP cleaving enzyme 1; PS1, presenilin-1; APP, amyloid precursor protein; GSH-Px, glutathione peroxidase; AChE, acetylcholinesterase; ChAT, choline acetyltransferase tumor necrosis factor-α (TNF-α), nuclear factor-kappaB (NF-κB)

Fig. 4. Putative mechanisms underlying cognitive impairment. The mechanisms identified in the figure represent those that could be modulated by moxibustion based on the reviewed studies.

in the brain.[50,51] Liu et al[33] reported the upregulation of choline acetyltransferase (ChAT) and GSH-Px and downregulation of AChE in the moxibustion group, suggesting that moxibustion could treat cognition loss by attenuating ACh deficits and mitochondrial oxidative stress.

4.2.2. *Controlling APP secretase and Aβ genesis*

Since Aβ is generated from β-secretase, including BACE-1 or γ-secretase composed of PS1 or PS2,[52,53] inhibiting γ- or β-secretase has been targeted for AD treatment.[54] Zhu et al[30] reported the downregulation of PS1 and BACE-1 after moxibustion treatment, indicating the effect of moxibustion in preventing Aβ genesis and other five studies also demonstrated reduced Aβ formation [26,28,30,33,55] by moxibustion treatment.

4.2.3. *Attenuating apoptosis*

Accumulated Aβ in AD and ischemia in VD are considered to induce massive neuronal apoptosis.[56,57] In response to apoptotic signals, overexpressed Bax promotes cell death by antagonizing the Bcl-2 complex.[58] Two studies[7,27] indicated the apoptosis-suppressive effect of moxibustion by demonstrating modulated Bcl-2 and Bax in the moxibustion group. Further, Weilan et al[38] reported the downregulation of C-fos protein and, two other studies[26,29] demonstrated the mitigated apoptotic morphology of cells after moxibustion treatment.

4.2.4. *Regulating inflammation*

In response to neuronal toxicity, activated microglia and astrocytes lead to increased levels of GFAP,[59,60] which is correlated with NFTs,[61,62] cytokines such as interleukin (IL) and tumor necrosis factor-α (TNF-α),[63] and pro-inflammatory proteins such as nuclear factor κB (NF-κb). IL-1β is genetically correlated with a high risk of AD,[64] IL-6 is elevated shortly after ischemic events, while IL-2 reduces amyloid plaque load.[65]

Jiang et al[31] proposed that moxibustion alleviated neuroinflammation based on findings of decreased IL-1β and increased IL-2 levels, which were consistent with the findings of Zhu et al,[30] Liu et al,[33] and Zhu et al[39] regarding attenuated pro-inflammatory factors after moxibustion treatment.[30,66]

4.2.6. *Modulating metabolites of TCA cycle and fatty acid metabolism*

Deficits in mitochondrial enzymes of the TCA are related to clinical disability in AD[67]; meanwhile lipid metabolism malfunction can contribute to the pathogenesis[68] of brain injuries and neuropsychiatric disorders.[69] Yu[32] observed that moxibustion increases the levels of metabolic products of the TCA cycle and fatty acid metabolism.

4.2.7. *Activating the VEGF*

In response to ischemia,[70] the angiogenic factor VEGF induces neuroprotective effects.[71] Therefore, decreased levels of VEGF cause chronic ischemia of neurons.[71] Three studies demonstrated that moxibustion treatment ameliorated ischemia-driven memory loss by improving levels of VEGF and basic fibroblast growth factor and its receptor. In addition, Zhang et al[36] reported the improved proliferation and migration of endothelial cells in moxibustion-treated VD rats (Fig. 4).

4.3. *Potential biases in the review process*

There are several limitations inducing potential biases in the review process. First, the low reporting quality reduced the credibility of the study results. Ambiguity in the model selection and outcome assessment presents a high risk of assessment bias, and insufficient reporting of excluded animals may indicate a high risk of reporting bias. Second, insufficient between-group baseline adjustments and differences in the therapeutic protocol could potentially distort the results, causing high heterogeneity between

studies. Third, the obtained results were insufficient to allow generalized conclusions regarding every possible cognitive impairment condition. The small number of included studies and different reporting formats impeded the analysis of some studies, and the included animal models do not encompass all possible cognitive impairment cases.

4.4. Comparison to previous reviews and implications

Compared to our previous SR[22] investigating the efficacy of moxibustion in preventing cognitive impairment, this study is more focused on the therapeutic effects of moxibustion. In both SRs, moxibustion treatment improved the behavioral test scores, and suppression of apoptosis and inflammation appeared to be the common mechanism induced by moxibustion. In preventive research, increased activity of neurotrophins, heat shock protein, and modulation of the cell cycle were demonstrated to be mediated by moxibustion; meanwhile, this study suggests modulation of metabolites and mitochondrial oxidative stress as the therapeutic mechanism of moxibustion.

4.5. Implication for clinical trials

In previous clinical studies, moxibustion treatment groups showed increased clinical scores accompanying attenuated metabolic factors including lower blood cholesterol levels,[72] suppressed oxidative stress,[17] and regulated balance between plasma thromboxane B2 and 6-keto-PG1α.[73] These findings have a part in common with the results of the present animal SR analysis. However, there is limited direct connection. The animal model design is inherently limited in terms of interpretation and application of human pathology. Unclear understanding of the pathology of human cognitive impairment makes it difficult to implement the results from an animal model in the treatment of humans.[74]

Despite the limitations, animal research has played a pivotal role in understanding the biological mechanisms of cognitive diseases and evaluating the efficacy of the drugs.[75] Although designing an ideal animal study and implementing a clinical study is challenging, interpreting research using various models and improved evaluation methods might be helpful in overcoming the limitations of biased analyses.

4.6. Conclusions

This SR showed that moxibustion might be beneficial in treating cognitive impairment. Its mechanism might encompass the suppression of oxidative stress and apoptosis, modulation of inflammation and Aβ genesis, enhancement of VEGF activity, and adjustment of metabolites of the TCA cycle and fatty acid metabolism. However, there were several limitations of this review, including the small number of included studies that lacked a common study design. Furthermore, the low reporting quality induced a high risk of bias and impeded the validation of the findings. More specific and rigorous trials with large sample sizes are needed to validate the efficacy of moxibustion for cognitive impairment and thoroughly examine the underlying mechanisms.

Author contributions

Sungmin Aum: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Seon Choe:** Formal analysis, Investigation. **Mudan Cai:** Writing - review & editing. **Ui Min Jerng:** Writing - review & editing, Supervision. **Jun-Hwan Lee:** Writing - review & editing, Supervision.

Conflicts of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; the collection, analyses, or interpretation of data; the writing of the manuscript; or in the decision to publish the results.

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Ethical statement

This research did not involve any human or animal experiment.

Data availability

The data will be made available upon reasonable request.

Supplementary material

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CRediT authorship contribution statement

Sungmin Aum: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Seon Choe:** Formal analysis, Investigation. **Mudan Cai:** Writing - review & editing. **Ui Min Jerng:** Writing - review & editing, Supervision. **Jun-Hwan Lee:** Writing - review & editing, Supervision.

References

1. Anekonda TS, Reddy PH. Can herbs provide a new generation of drugs for treating Alzheimer's disease? *Brain Res Brain Res Rev* 2005;50:361–76.
2. WHO. Mental health and older adults, <http://www.who.int/mediacentre/factsheets/fs381/en/>; Published 2016.
3. Yankner BA. Mechanisms of neuronal degeneration in alzheimer's disease. *Neuron* 1996;16:921–32.
4. Abramov AY, Duchen MR. The role of an astrocytic NADPH oxidase in the neurotoxicity of amyloid beta peptides. Philosophical transactions of the Royal Society of London Series B. *Prog Nucl Energy 6 Biol Sci* 2005;360:2309–14.
5. Ye Y, Zhu W, Wang XR, Yang JW, Xiao LY, Liu Y, et al. Mechanisms of acupuncture on vascular dementia—A review of animal studies. *Neurochem Int* 2017;107:204–10.
6. Piersanti M, Capannolo M, Turchetti M, Serroni N, De Berardis D, Evangelista P, et al. Increase in mortality rate in patients with dementia treated with atypical antipsychotics: A cohort study in outpatients in Central Italy. *Riv Psichiatr* 2014;49:34–40.
7. Xiao M. *Effect of expression and behavior of eight methods of Ling gui acupuncture moxibustion on AD on rat hippocampal Bax and Bcl-2 learning [Master degree]*. Guangxi University of Traditional Chinese Medicine; 2018.
8. Shen X, Ding G, Wei J, Zhao L, Zhou Y, Deng H, et al. An infrared radiation study of the biophysical characteristics of traditional moxibustion. *Complement Ther Med* 2006;14:213–9.
9. Xu X, Cao L, Mwandalima CJ, Wang Z, Liu L, Sun Z. Protocol for systematic review and meta-analysis: Moxibustion for treating ankylosing spondylitis. *Eur J Integr Med* 2017;12:142–6.
10. Kawakita K, Shinbara H, Imai K, Fukuda F, Yano T, Kuriyama K. How do acupuncture and moxibustion act? - focusing on the progress in Japanese acupuncture research. *J Pharmacol Sci* 2006;100:443–59.
11. Okada K., Kawakita K. Analgesic Action of Acupuncture and Moxibustion: A Review of Unique Approaches in Japan. 2007;6:11-17.
12. Ewies A, Olah K. Moxibustion in breech version—a descriptive review. *Acupunct Med* 2002;20:26–9.
13. Neri I, Airola G, Contu G, Allais G, Facchinetti F, Benedetto C. Acupuncture plus moxibustion to resolve breech presentation: A randomized controlled study. *J Matern Fetal Neonatal Med* 2004;15:247–52.
14. Yang Y, Wu XH. Study on the influence of metformin on castration-resistant prostate cancer PC-3 cell line biological behavior by its inhibition on

PLCepsilon gene-mediated Notch1/Hes and androgen receptor signaling pathway. *Eur Rev Med Pharmacol Sci* 2017;21:1918–23.
15. Tu JF, Yang JW, Lin LL, Wang TQ, Du YZ, Liu ZS, et al. Efficacy of electro-acupuncture and manual acupuncture versus sham acupuncture for knee osteoarthritis: Study protocol for a randomised controlled trial. *Trials* 2019;20:79.
16. Chen H, Wang P, Yang J, Liu G. Impacts of moxibustion on vascular dementia and neuropeptide substance content in cerebral spinal fluid. *Zhongguo Zhen jiu = Chinese Acupuncture & Moxibustion* 2011;31(1):19–22.
17. Ganghui L. Effects of combination of acupuncture and moxibustion with chinese drugs on lipid peroxide and antioxidase in patients of vascular dementia. *World J Acupunct Moxibustion* 1998.
18. Shao S, Tang Y, Guo Y, Tian Z, Xiang D, Wu J. Effects of acupuncture on patients with Alzheimer's disease: Protocol for a systematic review and meta-analysis. *Medicine* 2019;98:e14242.
19. Kwon CY, Lee B, Suh HW, Chung SY, Kim JW. Efficacy and safety of auricular acupuncture for cognitive impairment and dementia: a systematic review. *Evid Based Complement Altern Med* 2018;2018:3426078.
20. Hung CY, Wu X, Chung VC, Tang EC, Wu JC, Lau AY. Overview of systematic reviews with meta-analyses on acupuncture in post-stroke cognitive impairment and depression management. *Integr Med Res* 2019;8:145–59.
21. Leung MC, Yip KK, Ho YS, Siu FK, Li WC, Garner B. Mechanisms underlying the effect of acupuncture on cognitive improvement: A systematic review of animal studies. *J Neuroimmune Pharmacol* 2014;9:492–507.
22. Choe S, Cai M, Jerng UM, Lee JH. The efficacy and underlying mechanism of moxibustion in preventing cognitive impairment: A systematic review of animal studies. *Exp Neurobiol* 2018;27:1–15.
23. Macleod M, O'Collins T, Howells D, Donnan G. Pooling of animal experimental data reveals influence of study design and publication Bias. *Stroke; J Cereb Circ* 2004;35:1203–8.
24. CAMARADES, Published *Reporting of animal data from experimental studies*; 2014.
25. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biol* 2010;8:e1000412.
26. Du Y, Liu R, Sun G, Meng P, Song J. Pre-moxibustion and moxibustion prevent Alzheimer's disease. *Neural Regen Res* 2013;8:2811–9.
27. Wang S, Sun G, Ma J, Li X, Wan B. Effects of moxibustion on behaviors and Bcl-2, Bax, Caspase-3 in Hippocampus of alzheimer's disease model rats. *World Sci Technol?Modern Tradit Chin Med Materia Medica* 2015;17: 1243–8.
28. Wang S, Ma J, Sun G, Shen F, Li X, Wan B. Effect of moxibustion on morphology of Hippocampus in alzheimer' s disease model rats. *Chin J Gerontol* 2015;36:4993–5.
29. Wang S, Sun G, Ma J, Li X, Wan B. Effects of moxibustion on behaviors and hippocampal ultrastructure of alzheimer's disease model rats. *Chin J Inform TCM* 2015;22:58–61.
30. Zhu C, Jian J, Han W, Yang J. Effect of moxibusiton on learning-memory ability and hippocampal Amyloid β protein overexpression in Mild Cognitive Impairment rats. *Acupunct Res* 2016;41:131–7.
31. Jiang M, Liang J, Wang J, et al. Effect of Moxibustion on the Learning and Memory of Rat Models of Alzheimer's Disease and the Expression of Hippocampal Aβ, IL-1β and IL-2. *Shanghai J Acu-mox* 2016;35:870–5.
32. Yu M. *Metabolomics study of urine and plasma targets in APP/PS1 double transgenic AD mice by moxibustion and Aiwu [master's degree]*. Beijing University of Chinese Medicine; 2017.
33. Liu J, Zhao B, Cui Y, Huang Y, Huang C, Huang J, et al. Effects of shenque moxibustion on behavioral changes and brain oxidative state in apolipoprotein e-deficient mice. *Evid Based Complement Altern Med* 2015;2015.
34. Wang PY, Jun Z, Qing P. Moxibustion head acupoints on vascular dementia rats affection of VEGF and its receptor Flt-1, Flk-1mRNA expression. *J Emerg Tradit Chin Med* 2013.
35. Wang M, Qing P, Wu S, Zhang F, Ma Z, Zhang Y, et al. Effect of Huayu Tongluo Moxibustion on the Ultrastructure of the Frontal of SVD Rats. *J Clin Acupunct Moxibust* 2015;31:59–61.
36. Zhang M, Yang J, Wang P, Wang P, editors. The Effect of Stasis-eliminating and Meridian-unblocking Moxibustion on Vascular Endothelial Cell Proliferation and Migration in VD Rats. 2017 World Acupuncture Academic Conference and 2017 China Acupuncture Association Annual Meeting; 2017; Beijing China.
37. Wang P, Tang JY, Yang J. Effects of moxibustion on the expressions of hippocampal VEGF, flt-1, bFGF, and bFGF-r in vascular dementia rats. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2012;32:97–101.
38. Weilan Q, Yan Y, Xuemei L, Wei S, Xiaohan L, Qingke M, et al. Effect of medicated thread moxibustion on apoptosis of hippocampal neurons in rat models of chronic cerebral ischemic vascular dementia. *Cell Mol Biol (Noisy-le-grand)* 2018;64:107–12.
39. Zhu W, Yang K, Cai S, Wang Y, Song X. Effect of warming yang and supplementing kidney moxibustion therapy on the NF-κB signaling pathway of vascular dementia rats. *World J Acupunct - Moxibustion* 2018;28:44–9.
40. Luo B, Guo Y, Peng M, Zhang Y, Wang Y, Qi B. The effect of "Yiqi Tiaoxue, Fuben Peiyuan" medicine moxibustion on spatial memory of rats with multiple infarct dementia. 2018;38:2718-20.
41. Yu J, Jia M, Gao L, Zhang, Yong S, Bao Y. Effects of grain-sized moxibustion on learning and memory ability and amyloid deposition of transgenic Alzheimer's desease mice. Acupunct Research 2014;39.
42. Bao Y, Zhang Y, Chu J, et al. Effects of grain-sized moxibustion on expression of Aβ1-42 in prefrontal cortex and hippocampus in double-transgenic AD mice. *Chinese Acupuncture & Moxibustion* 2015;35(1):59–65.
43. Liu R. *The effect of preventive moxibustion on expression of 14-3-3 protein and apoptosis of hippocampal neurons in rats of Alzheimer's disease*. Hubei university of Chinese medicine: Hubei university of Chinese medicine; 2012.
44. Kim MH, Seo JY, Han MK, Kang HR, Kim SS, Kim BR, et al. Chronic administration of Artemisia annua L. Attenuates oxidative stress induced by D-galactose in mice. *FASEB J* 2014;28.
45. Li J. *The experimental study of the expression of Pre-stimulation with moxibustion on CDK5, P27kip1 in rats of Alzheimer's disease*. Hubei University of Chinese Medicine: Hubei University of Chinese Medicine; 2011.
46. Li X. *Research on the action mechanism of p38MAPK signal pathway in treating the rats with Alzheimer disease by preventative moxibustion*. Hubei University of Chinese Medicine: Hubei University of Chinese Medicine; 2012.
47. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989;20:84–91.
48. Khan MB, Khan MB, Khan A, Ahmed ME, Ishrat T, Tabassum R, et al. Naringenin ameliorates Alzheimer's disease (AD)-type neurodegeneration with cognitive impairment (AD-TNDCI) caused by the intracerebroventricular-streptozotocin in rat model. *Neurochem Int* 2012;61:1081–93.
49. Martin JB. Molecular basis of the neurodegenerative disorders. *N Engl J Med* 1999;340:1970–80.
50. Ejaz Ahmed M, Khan MM, Javed H, Vaibhav K, Khan A, Tabassum R, et al. Amelioration of cognitive impairment and neurodegeneration by catechin hydrate in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. *Neurochem Int* 2013;62:492–501.
51. Neha, Sodhi RK, Jaggi AS, Singh N. Animal models of dementia and cognitive dysfunction. *Life Sci* 2014;109:73–86.
52. Mirra SS, Hart MN, Terry RD. Making the diagnosis of Alzheimer's disease. A primer for practicing pathologists. *Arch Pathol Lab Med* 1993;117:132–44.
53. O'Brien RJ, Wong PC. Amyloid precursor protein processing and alzheimer's disease. *Annu Rev Neurosci* 2011;34:185–204.
54. Heneka MT, Carson MJ, Khoury JE, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015;14:388–405.
55. Wever KE, Menting TP, Rovers M, van der Vliet JA, Rongen GA, Masereeuw R, et al. Ischemic preconditioning in the animal kidney, a systematic review and meta-analysis. *PLoS One* 2012;7:e32296.
56. Wang X, Wu J, Yu C, Tang Y, Liu J, Chen H, et al. Lychee seed saponins improve cognitive function and prevent neuronal injury via inhibiting neuronal apoptosis in a rat model of alzheimer's disease. *Nutrients* 2017;9.
57. Shimohama S. Apoptosis in Alzheimer's disease—an update. *Apoptosis* 2000;5:9–16.
58. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993;74:609–19.
59. Kato S, Gondo T, Hoshii Y, Takahashi M, Yamada M, Ishihara T. Confocal observation of senile plaques in Alzheimer's disease: Senile plaque morphology and relationship between senile plaques and astrocytes. *Pathol Int* 1998;48:332–40.
60. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia* 2005;50:427–34.
61. Vehmas AK, Kawas CH, Stewart WF, Troncoso JC. Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. *Neurobiol Aging* 2003;24:321–31.
62. Kamphuis W, Middeldorp J, Kooijman L, Sluijs JA, Kooi E-J, Moeton M, et al. Glial fibrillary acidic protein isoform expression in plaque related astrogliosis in Alzheimer's disease. *Neurobiol Aging* 2014;35:492–510.
63. Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Ann Transl Med* 2015;3:136.
64. Swardfager W, Lanctot K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry* 2010;68:930–41.
65. Alves S, Churlaud G, Audrain M, Michaelsen-Preusse K, Fol R, Souchet B, et al. Interleukin-2 improves amyloid pathology, synaptic failure and memory in Alzheimer's disease mice. *Brain* 2017;140:826–42.
66. Zhang H, Zhou S, Song X, Xu W, Li L, Su S, et al. Regulating effect of moxibustion on MCP-1 and NF-κB in the colonic mucosa tissue of rats with Crohn's disease. *World J Acupunct - Moxibustion* 2016;26:33–40.
67. Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE. Mitochondrial abnormalities in Alzheimer brain: Mechanistic implications. *Ann Neurol* 2005;57:695–703.
68. Wong MW, Braidy N, Poljak A, Pickford R, Thambisetty M, Sachdev PS. Dysregulation of lipids in Alzheimer's disease and their role as potential biomarkers. *Alzheimer Demen* 2017;13:810–27.
69. Hussain G, Anwar H, Rasul A, Imran A, Qasim M, Zafar S, et al. Lipids as biomarkers of brain disorders. *Crit Rev Food Sci Nutr* 2019:1–24.
70. Svensson B, Peters M, Konig HG, Poppe M, Levkau B, Rothermundt M, et al. Vascular endothelial growth factor protects cultured rat hippocampal neurons against hypoxic injury via an antiexcitotoxic, caspase-independent mechanism. *J Cereb Blood Flow Metab* 2002;22:1170–5.
71. Storkebaum E, Carmeliet P. VEGF: A critical player in neurodegeneration. *J Clin Invest* 2004;113:14–8.
72. Wang P, Yang J, Liu G, Chen H, Yang F. Effects of moxibustion at head-points on levels of somatostatin and arginine vasopressin from cerebrospinal fluid in

patients with vascular dementia: a randomized controlled trial. *Chin J Tradit Chin Med Phar* 2010;8:636–40.
73. Liang Y. Effect of acupuncture-moxibustion plus chinese medicinal herbs on plasma TXB2, 6-Keto-PGF1α in patients with vascular dementia. *World J Acupunct Moxibustion* 1998;23(2):17–9.
74. Götz J, Bodea LG, Goedert M. Rodent models for Alzheimer disease. *Nat Rev Neurosci* 2018;19:583–98.
75. Goodarzi P, Payab M, Alavi-Moghadam S, Larijani B, Rahim F, Bana N, et al. Development and validation of alzheimer's disease animal model for the purpose of regenerative medicine. *Cell Tissue Bank* 2019;20:141–51.