Published online 2022 May 20 **Original Article**

Effect of Curcumin on Hippocampal Neurons, Learning, and Spatial Memory in a Model of Global Cerebral Ischemia

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Received 2021 October 16; **Revised** 2021 December 17; **Accepted** 2022 March 22.

Abstract

Background: Curcumin is a natural antioxidant known for its neuroprotective properties against cerebral ischemic reperfusion injury. Stroke has the greatest impact on the hippocampus cornu ammonis (CA1) region and leads to cognitive impairment.

Objectives: This study aimed to investigate the effect of pretreatment with turmeric extracts before the induction of cerebral ischemia on the structure of the hippocampus CA1 region, learning, and spatial memory.

Methods: A total of 32 adult male Wistar rats were randomly divided into four groups: 1) control group, 2) sham group (received dimethyl sulfoxide), 3) ischemia group (15 min bilateral carotid artery), and 4) curcumin group (received 100 mg/kg of curcumin daily for five days before the induction of ischemia). The Morris water maze test was performed to evaluate memory impairment in mice 48 h after ischemia induction. The animals were then anesthetized, and their blood samples were taken for malondialdehyde measurement. Subsequently, the animals' brains were removed, and the number of neurons, as well as the volume of the layers, were examined using cresyl violet staining.

Results: The results of the stereology showed a significant increase in the volume of the CA1 region and its substrates in the curcumin treatment group, compared to the ischemia group. In addition, the number of pyramidal neurons in the treatment group showed a significant increase, in comparison with the ischemia group. Moreover, curcumin administration reduced the spatial memory impairment in the treatment group, compared to the ischemia group.

Conclusion: These findings suggest that pretreatment with curcumin can improve memory and learning disorders, as well as hippocampal neuron damage, following ischemia.

Keywords: Curcumin, Hippocampus, Ischemia, Stereology

1. Background

Stroke is the most common cause of disability in adults worldwide. Due to causing disability and many other complications, it has become a serious threat to human health and the quality of life (1, 2). The most important pathological events during ischemia include the depletion of cellular energy reserves due to the decreased blood flow, dysfunction of the ion pumps, hypoxic depolarization, intracellular acidosis, increased sodium and calcium, releasing amino acids, as well as the stimulation of nitric oxide production (3). These conditions then lead to motor-sensory and vision disorders, speech disorders, as well as neuropsychological disorders, such as decreased perception, cognitive disorders, learning disabilities, anterograde amnesia, and the inability to perform previously learned tasks (4).

Numerous studies in the past have indicated that the blood flow return after ischemia has the greatest effect on brain tissue (5-7). The hippocampus is part of the temporal lobe that plays a key role in memory stabilization and learning (8). The cornu ammonis (CA1) region is the most complex part of the hippocampus, whose neurons receive the most damage after reperfusion-ischemia injury. During reperfusion, free radicals attack brain tissue, causing neuronal necrosis and apoptosis through a series of processes, such as impaired energy metabolism, inflammation, oxidative damage, and calcium overload (9-11). Lipid peroxidation has led to toxic aldehydes, one of the most toxic of which is malondialdehyde (MDA) currently considered an indicator of lipid peroxidation. Therefore, measuring MDA can determine the damage of cellular lipids against oxidation (12).

Previous studies have proven that a timely treatment with neuroprotective medications can reduce apoptosis and release superoxide-free radicals (13).

Curcumin is the main ingredient of the turmeric plant, produced from the rhizome of Curcuma longa, an ancient plant that belongs to the Zingberasia family. In addition, it was used in ancient India as an effective treatment for many diseases (14). Curcumin exhibits anti-inflammatory, anti-cancer and antioxidant activities, as well as several other medicinal properties (15). The neuroprotective effects of curcumin result from its antioxidant potential reported in previous studies (16). Curcumin

crosses the blood-brain barrier and has the potential to inhibit the formation of Amyloid Beta Oligomers and apoptosis. Therefore, using it is recommended for the prevention or treatment of cerebral ischemia and Alzheimer's disease (17,18).

2. Objectives

The majority of previous studies on stroke complications were molecular cell studies counting the number of neurons. For this reason, stereology was used in this study to examine the total volume and different areas of the CA1 region, the total number of cells in the pyramidal layer CA1, learning, and spatial memory.

3. Methods

3.1. Animal experiment

This experimental study was performed on 32 male Wistar rats with an average weight of 250-300 g purchased from Yazd University of Medical Sciences (Yazd, Iran). This study was carried out with the approval of the Medical Ethics Committee of Yazd University of Medical Sciences (Registration number: IR.SSU.MEDICINE.REC.1399.091) and according to the Core Principles for the Care and Use of Animals in Research. The animals were kept under standard conditions with a 12:12 light-dark cycle at 21±3˚C and had full access to adequate drinking water, as well as food. They were randomly selected and divided into four groups: control, sham, ischemia, and treatment. The control group (n=8) was evaluated without any intervention. The sham group (n=8) only underwent anesthesia and surgery. In the ischemia-reperfusion group (n=8), bilateral carotid arteries were closed. The last group was the curcumin treatment group 100 mg/kg+Ischemia-Reperfusion (n=8). The dose of curcumin was determined based on previous experiments (19).

3.2. Medicine treatment

A total of 100 mg of curcumin (Sigma, USA) was dissolved in 1 ml of dimethyl sulfoxide (DMSO) (Sigma, USA). In the treatment group, mice were treated with 100 mg/kg of curcumin intraperitoneally for five days before the onset of obstruction (19). The sham group was also injected with the same volume of DMSO intraperitoneally.

3.3. Development of transient ischemic attack

The Bilateral Common Carotid Artery Occlusion model was performed according to previous studies (20). The animals were first anesthetized by 100 mg/kg of ketamine and 10 mg/kg of xylazine intraperitoneal injection. The common carotid arteries were occluded for 15 min with a longitudinal incision of 1 cm below the neck on each side (21).

3.4. Morris water maze test

Forty-eight hours after the ischemia-reperfusion induction, the Morris water maze (MWM) test was run to evaluate memory impairment. The MWM is made up of an iron circular pool (diameter 100 cm, height 50 cm, painted in black) that is filled to a depth of 30 cm with water. A circular transparent hidden platform (10 cm in diameter) sunk approximately 1 cm below the water surface and 10 cm from the edge of the pool, at the target quarter location. For trial-and-error learning, the platform was retained in the same place, and during the probing test, it was withdrawn from the pool. The mouse had to swim to find the escape platform and stay on it for 20 sec. Time and distance traveled from the beginning to the time of climbing the platform were recorded as the latency. Furthermore, the camera filmed the distance, and the information was recorded by the water maze software (Borj Sanat Azma, Iran) (22).

3.5. Serum malondialdehyde assay

The animals were anesthetized, and their blood samples were taken. The samples were centrifuged at 3,000 rpm for 10 min. After the serum was separated, a special MDA kit was used to measure MDA. The wavelength was then measured and recorded in the 535 spectra by the extinction coefficient for MDA (23).

3.6. Brain tissue processing

After cutting out the brain, to prepare tissue sections from the mouse hippocampus, brain hemispheres were rinsed with running water for 15 min, and then, they were dehydrated with alcohol and clarified with xylol, as well as paraffin impregnation. Finally, the samples were placed in special cassettes of a tissue processor. After molding by a German microtome (Leitz 1512), 5-µm slices were prepared and examined by 1% cresyl violet (Sigma-Aldrich, USA) staining (24). From each sample, 10 sections were selected to be stained with cresyl violet (Figure 1).

3.7. Stereology

3.7.1. Tissue volume calculation

The Cavalier technique was used to determine the total volume of the CA1 region and its sublayers. In this method, a point-counting grid was utilized to calculate the volume. Tissue cutting was performed serially with a thickness of 5 µm at a fixed distance of 250 µm, and photography was performed using a light microscope under a 4× lens (Olympus, Japan) with a final magnification of 40%. An average of 7 to 10 sections were selected from each tissue sample to count the volume of the CA1 region. Finally, to calculate the volume of the CA1 region and sublayers, the following formula was used (25).

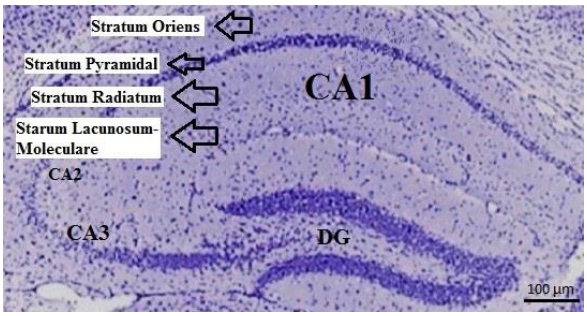

Figure 1. Different substrates of the cornu ammonis region (40× cresyl violet staining)

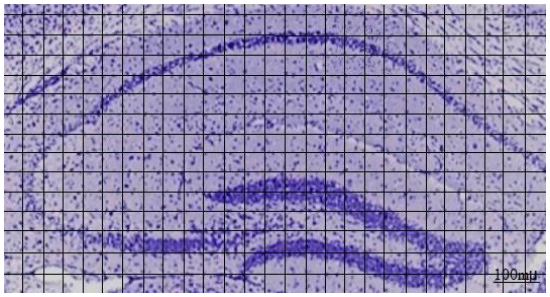

Figure 2. Calculating the volume of the cornu ammonis region and its substrates by the Cavalier method (cresyl violet staining 40×)

$$V_{ref} = \frac{\sum pi \times A(pi) \times t}{M^2}$$

Where (ΣP) is the total number of points, t is the thickness of the slices, A is the area around each point, and the magnification is denoted by M2 (Figure 2).

3.7.2. Calculation of numbers in stereology

The physical disector method was employed to calculate the number of CA1 pyramidal cells. In this method, two consecutive sections were used, and only the nuclei of cells in the reference box were counted, not those in the search box (Figure 3). Finally, on average, 17 to 20 disectors were used for each sample (26). The dimensions of each disector were 20×20 mm, and photography was performed using an objective lens of 40 and a final magnification of 400 in each section. Finally, numerical density was obtained from the following equation.

$$Nv = \sum Q / N(dis) \times V(dis)$$

Where, $\sum Q$ is the sum of cells counted, Nv shows the density, N (disc) represents the number of dissectors, and V shows the volume of the disectors.

If the numerical density is multiplied by the reference volume, the total number of cells is obtained from the following equation.

$$N = N_V \times V_{ref}$$

3.8. Statistical analysis

After data collection, data were analyzed using the SPSS software (version 19). Data obtained from the MWM test were evaluated through the Two-Way repeated-measures and One-Way ANOVA while other data were assessed using the Tukey HSD test. Differences were considered significant with P≤0.05. The normal distribution of data was also assessed by the Kolmogorov-Smirnov test.

4. Results

4.1. Stereology

The comparison of the mean values of different CA1 layers in the hippocampus of the studied rats showed that the volume of different CA1 layers in the ischemia group was significantly different from other groups (P<0.05). Although the volume of sublayers in the sham and treatment groups differed from that of the control group, this difference was not significant (Table 1).

The mean numerical density of the pyramidal neurons in the CA1 region per mm3 of the hippocampus was compared across the studied groups, showing that the number of neurons in the pyramidal layer of the ischemia group (receiving no medications) decreased, compared to that in other groups. This difference was significant with the control group (P<0.001), the sham group (P<0.001), and the treatment group (P<0.004) (Table 2).

The examination of serum by the ELISA test showed that the amount of MDA in the treatment group was significantly reduced, in comparison with the medication-free ischemia group (P<0.001). The difference between the control group, the sham group, and the treatment group was significant. The MDA concentrations in different groups are shown as mean±SE in µM (Table 3).

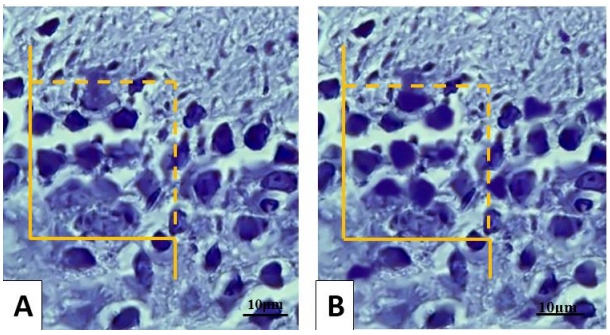

Figure 3. Physical disector grid showing included lines (continuous line) and forbidden lines (dotted line) A: the reference section, B: the look-up section (cresyl violet staining, 400×)

Table 1. Mean±SE volume of the layers of the hippocampal CA1 field (mm^3) in the experimental groups

Group	Stratum Oriens	Stratum Pyramidal	Stratum Radiatum+Lacunosum Molecular	CA1
C	2.56±0.025	1.62±0.052	6.13±0.031	10.32±0.025
SH	2.52±0.044	1.59±0.004	6.06±0.051	10.19±0.008
I/R	3.72±0.031*	1.78±0.007**	6.76±0.092***	11.27±0.109 ****
CUR	2.59±0.016	1.66±0.007	6.25±0.021	10.50±0.033

Results of the Two-Way ANOVA *with the Tukey HSD post hoc test*
*Significant difference with the control, sham, and treatment groups (P<0.001)
**Significant difference with the control group (P<0.012), sham group (P<0.002), and treatment group (P<0.043)
***Significant difference with the control group (P<0.002), sham group (P<0.001), and treatment group (P<0.015)
****Significant difference with the control, sham, and treatment groups (P<0.001).
Cornu Ammonis (CA1), Control (C), Sham (SH), Ischemia/reperfusion (I/R), Ischemia+Curcumin (CUR)

Table 2. Mean±SE of the total number of pyramidal neurons in experimental groups ($\times 10^3$)

Group	N	CA1
C	8	185±7
SH	8	174±5
I/R	8	143±2 #*
CUR	8	170±1

Results of the Two-Way ANOVA with the Tukey HSD post hoc test
#Significant difference with the control and sham groups (P<0.001)
*Significant difference with the treatment group (P<0.004)
Cornu Ammonis (CA1), Control (C), Sham (SH), Ischemia/reperfusion (I/R), Ischemia+Curcumin (CUR)

Table 3. Mean±SE level of serum MDA (µM) after brain ischemia

Group	N	MDA
C	8	4.020±0.13
SH	8	4.611±0.31
I/R	8	8.646±0.27*
CUR	8	5.109±0.33

Results of the One-Way ANOVA with the Tukey HSD post hoc test
*Significant difference with the control, sham, and treatment groups (P<0.001).
Malondialdehyde (MDA), Control (C), Sham (SH), Ischemia/reperfusion (I/R), Ischemia + Curcumin (CUR)

4.2. Morris water maze

The examination of the data obtained from the MWM experiment showed that the distance traveled by animals decreased during four consecutive days. The results obtained from comparing the distance traveled across the studied groups show a significant difference between the treatment group and the ischemia group in that the ischemia group traveled longer distances to reach the platform than the other groups (P<0.001). The treatment group also traveled longer distances than the control and sham groups, but this difference was not statistically significant (Figure 4). Control (C), Sham (SH), Ischemia/reperfusion (I/R), Ischemia+Curcumin (CUR).

Analysis of the data on the time taken to find the platform across the groups revealed that the time to reach the platform decreased in all groups. The treatment group had a significant decrease, compared to the ischemia group (P<0.05). Animals in the ischemia group spent more time reaching the platform on all experiment days than the control and sham groups, which was a significant difference (P<0.05). Although animals in the treatment group spent more time than the control group, this increase was insignificant (Figure 5).

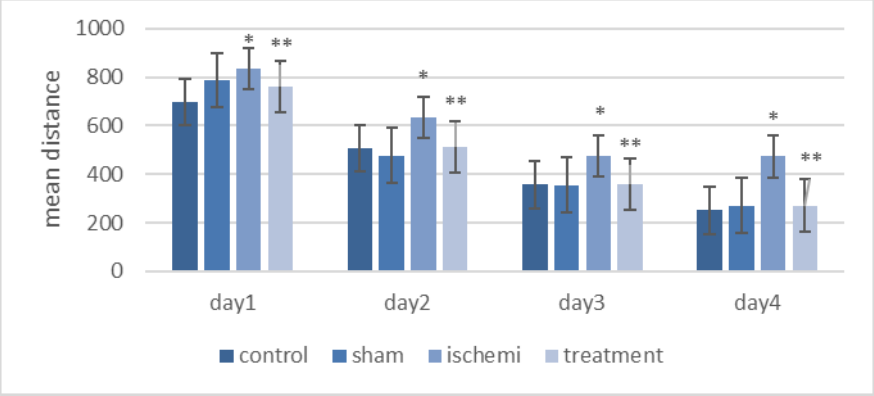

Figure 4. Traveled distance in the Morris water maze test (mean±SE, n=8) in different groups. Statistical analysis was performed using the Two-Way analysis of variance (ANOVA) with repeated measures.
*Significant difference with the control, sham, and treatment groups (P<0.001)
**Significant difference with the ischemia/reperfusion group (P<0.001)

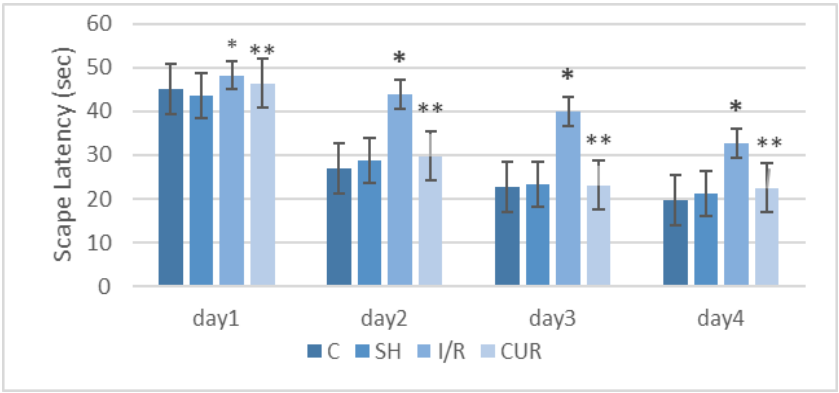

Figure 5. Escape latency time (sec) in the Morris water maze test (mean±SE, n=8) in different groups
*Significant difference with the control, sham, and treatment groups (P<0.05)
**Significant difference with the ischemia/reperfusion group (P<0.05)
Control (C), Sham (SH), Ischemia/reperfusion (I/R), Ischemia+Curcumin (CUR)

The results of the fifth day indicated that animals in the ischemia group traveled less distance in the target quadrant than the other groups and that there was a significant difference between the ischemia group and the other groups (P<0.001) (Figure 6).

The ischemia group also spent less time in the target quadrant than the other groups (P<0.001) (Figure.7).

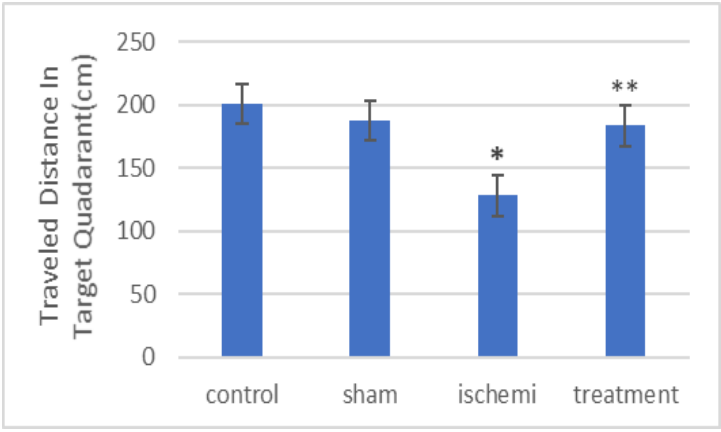

Figure 6. Traveled distance in the target quadrant (mean±SE, n=8) in different groups
* Significant difference with control, sham, and treatment groups (P<0.001)
** Significant difference with the ischemia/reperfusion group (P<0.001)

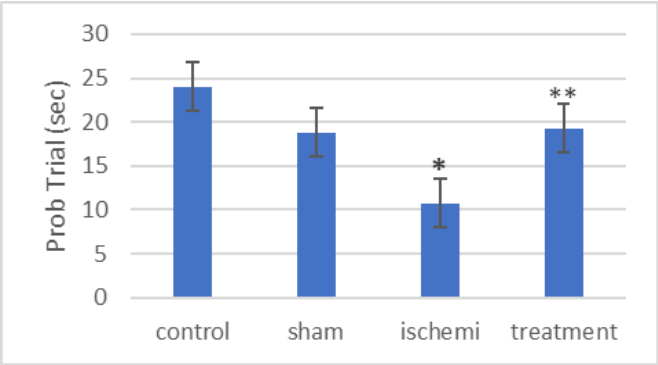

Figure 7. Time (sec) spent in the target (mean±SE, n=8) in the different groups.
*Significant difference with the control, sham, and treatment groups (P<0.05)
**Significant difference with the ischemia/reperfusion group (P<0.05)

5. Discussion

The present study investigated the effect of administering curcumin before the induction of cerebral ischemic reperfusion injury (CRIR) on the stereological parameters of the CA1 hippocampal region, learning, and spatial memory in male Wistar rats. The findings indicated that the administration of curcumin at a dose of 100 mg/kg five days before the induction of ischemic injury improved male rats' learning parameters and memory. In addition, curcumin was found to increase the numerical density of pyramidal cells in the treatment group relative to the ischemia-reperfusion group and decrease the amount of MDA in the treatment group, compared to the ischemia group.

In reperfusion ischemia, the return of blood flow to the tissue causes inflammation, oxidative stress, increased MDA, tissue necrosis, apoptosis, and the overproduction of Reactive Oxygen Species (ROS). Previous reports have shown that transient cerebral ischemia stimulates lipid peroxidation and reduces the antioxidant power of the brain (27,28). Additionally, in the study of Fadhel et al., after ischemia, a significant increase was observed in the level of MDA of the hippocampus with a decrease in Glutathione (29). As a result of these mechanisms, a decrease in the number of neurons indicated cerebral ischemia and reperfusion damage due to the overproduction of ROS, which causes oxidative stress. The inflammation and cerebral edema phenomenon that occur during ischemia-reperfusion (30) can explain the increase in ischemia-induced volume in the CA1 region and its sublayers. Hence, the findings of the present study confirmed that extensive tissue damage is directly related to the induction of oxidative stress.

Curcumin can protect the brain against ischemic injury. It has been shown to significantly prevent CIRI through mechanisms that improve oxidative damage (31,32). Another study reported that curcumin reduced ischemic oxidative damage in rats after stroke (32). Curcumin also promotes the expression of thioredoxin (an antioxidant protein) and protects neurons from oxygen-glucose deprivation death in the ischemia-reperfusion model (33). In the present study, curcumin use could reduce neuronal damage and improve ischemia-induced memory impairment, as well as learning disorders, confirming the findings of previous studies.

The results of the present study showed that the amount of MDA was significantly reduced in the curcumin group, compared to the ischemia group, which indicates a reduction in lipid peroxidation, inflammation, and tissue damage. The extent of inflammation after stroke was assessed by YanpingMiao et al. Their observations showed that curcumin protects the brain against ischemia by suppressing inflammatory cytokines (34). Another study reported that curcumin could reduce the MDA of brain tissue after ischemia (30). The findings of the present study also revealed that curcumin could lead to neuroprotection by reducing lipid peroxidation, which is consistent with the results of previous studies.

The results of previous studies on different models of stroke revealed that curcumin exerts its neuroprotective function through its antioxidant properties, the regulation of cellular apoptosis, and increased neurogenesis (35). This property can be considered a key indicator for preventing neuronal damage and destruction. According to previous reports, pretreatment with curcumin can improve nerve function and reduce the lesion caused by cerebral ischemia (36). Chen et al. (2017) showed that curcumin has a protective effect against neuronal apoptosis after cerebral ischemia (37). In another study, the consumption of curcumin at a dose of 100 mg/kg could reduce ischemia-induced apoptotic neuronal cell death in the hippocampus (38). The neuroprotective effect of curcumin has been reported in several studies of cerebral ischemia (39,40). In the present study, curcumin improved neuronal density, which may be related to its antioxidant properties.

The decrease of neurons in the hippocampus is one of the most important signs of impaired memory

and learning (41). Since there are pyramidal cells in the CA1 region of the hippocampus that play an essential role in the formation of memory and learning and are also the most vulnerable regions during ischemia, memory and learning disorders can be attributed to the reduced pyramidal neurons after cerebral ischemia-reperfusion.

Several studies have shown that curcumin can improve memory and spatial learning in Wistar rats (39,42). Curcumin pretreatment improves impaired spatial working memory by inhibiting proinflammatory cytokines in rats undergoing cerebral ischemia and reperfusion (43). This study also showed that curcumin improves memory and learning disorders caused by ischemia, which is similar to previous results.

6. Conclusion

In general, the results of this study revealed that the administration of curcumin before stroke reduces cell death, neuronal damage, inflammatory factor MDA, as well as memory and learning disorders. As a result, long-term use of curcumin products in high amounts may prevent neuronal damage and reduce inflammation from stroke, thereby significantly reducing memory and learning disorders.

Acknowledgments

The authors would like to thank all the members of the Department of Anatomical Sciences at Yazd University of Medical Sciences, Yazd, Iran.

Footnotes

Conflicts of Interest: The authors declare no conflicts of interest.
Authors' contributions: All authors contributed equally to the research design, conduction of the experiment, analysis of the results, and preparation of the manuscript.
Financial support: This study was supported by the Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

References

1. Hankey GJ. Stroke. *Lancet*. 2017;**389**(10069):641-54. doi: 10.1016/S0140-6736(16)30962-X. [PubMed: 27637676].
2. 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**(4):e38-360. doi: 10.1161/CIR.0000000000000350. [PubMed: 26673558].
3. Fisher M. The spectrum of translational stroke research. *Neurol Res*. 2013;**35**(5):443-7. doi: 10.1179/1743132813Y.0000000214. [PubMed: 23594388].
4. Nadia Sharifi Z, Abolhassani F, Hassanzadeh G, Zarrindast MR, Movassaghi S. Neuroprotective treatment with FK506 reduces hippocampal damage and prevents learning and memory deficits after transient global ischemia in rat. *Arch Neurosci*. 2014;**1**(1):35-40. doi: 10.5812/archneurosci.9163.
5. Chen YW, Sung SF, Chen CH, Tang SC, Tsai LK, Lin HJ, et al. Intravenous thrombolysis administration 3–4.5 h after acute ischemic stroke: A retrospective, multicenter study. *Front Neurol*. 2019;**10**:1038. doi: 10.3389/fneur.2019.01038. [PubMed: 31681138].
6. 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**(12):344-418. doi: 10.1161/STR.0000000000000211. [PubMed: 31662037].
7. Qureshi AI, Singh B, Huang W, Du Z, Lobanova I, Liaqat J, et al. Mechanical thrombectomy in acute ischemic stroke patients performed within and outside clinical trials in the United States. *Neurosurgery*. 2020;**86**(1):2-8. doi: 10.1093/neuros/nyz359. [PubMed: 31670379].
8. Wang Z, Ye Z, Huang G, Wang N, Wang E, Guo Q. Sevoflurane post-conditioning enhanced hippocampal neuron resistance to global cerebral ischemia induced by cardiac arrest in rats through PI3K/Akt survival pathway. *Front Cell Neurosci*. 2016;**10**:271. doi: 10.3389/fncel.2016.00271. [PubMed: 27965539].
9. Chen CH, Hsieh CL. Effect of acupuncture on oxidative stress induced by cerebral ischemia-reperfusion injury. *Antioxidants*. 2020;**9**(3):248. doi: 10.3390/antiox9030248. [PubMed: 32204376].
10. Khandelwal P, Yavagal DR, Sacco RL. Acute ischemic stroke intervention. *J Am Coll Cardiol*. 2016;**67**(22):2631-44. doi: 10.1016/j.jacc.2016.03.555. [PubMed: 27256835].
11. Wu L, Xiong X, Wu X, Ye Y, Jian Z, Zhi Z, et al. Targeting oxidative stress and inflammation to prevent ischemia-reperfusion injury. *Front Mol Neurosci*. 2020;**13**:28. doi: 10.3389/fnmol.2020.00028. [PubMed: 32194375].
12. Singleton RH, Yan HQ, Fellows-Mayle W, Dixon CE. Resveratrol attenuates behavioral impairments and reduces cortical and hippocampal loss in a rat controlled cortical impact model of traumatic brain injury. *J Neurotrauma*. 2010;**27**(6):1091-9. doi: 10.1089/neu.2010.1291.[PubMed: 20560755].
13. Zhang Y, Zhang Y, Jin Xf, Zhou Xh, Dong Xh, Yu WT, et al. The role of astragaloside IV against cerebral ischemia/reperfusion injury: suppression of apoptosis via promotion of P62-LC3-autophagy. *Molecules*. 2019;**24**(9):1838. doi: 10.3390/molecules24091838. [PubMed: 31086091].
14. Li H, Sureda A, Devkota HP, Pittalà V, Barreca D, Silva AS, et al. Curcumin, the golden spice in treating cardiovascular diseases. *Biotechnol Adv*. 2020;**38**:107343. doi: 10.1016/j.biotechadv.2019.01.010. [PubMed: 30716389].
15. Khurana S, Jain S, Mediratta PK, Banerjee BD, Sharma KK. Protective role of curcumin on colchicine-induced cognitive dysfunction and oxidative stress in rats. *Hum Exp Toxicol*. 2012;**31**(7):686-97. doi: 10.1177/0960327111433897.
16. Merrell JG, McLaughlin SW, Tie L, Laurencin CT, Chen AF, Nair LS. Curcumin loaded poly (ε-caprolactone) nanofibers: diabetic wound dressing with antioxidant and anti-inflammatory properties. *Clin Exp Pharmacol Physiol*. 2009;**36**(12):1149-56. doi: 10.1111/j.1440-1681.2009.05216.x. [PubMed: 19473187].
17. Wang Y, Yin H, Wang L, Shuboy A, Lou J, Han B, et al. Curcumin as a potential treatment for Alzheimer's disease: a study of the effects of curcumin on hippocampal expression of glial fibrillary acidic protein. *Am J Chinese Med AM J*. 2013;**41**(01):59-70. doi: 10.1142/S0192415X13500055.
18. Wu L, Jiang C, Kang Y, Dai Y, Fang W, Huang P. Curcumin exerts protective effects against hypoxia‐reoxygenation injury via the enhancement of apurinic/apyrimidinic endonuclease 1 in SH‐SY5Y cells: Involvement of the PI3K/AKT pathway. *Int J Med*. 2020;**45**(4):993-1004. doi: 10.3892/ijmm.2020.4483. [PubMed: 32124937].
19. Liu L, Zhang W, Wang L, Li Y, Tan B, Lu X, et al. Curcumin prevents cerebral ischemia reperfusion injury via increase of mitochondrial biogenesis. *Neurochem Res*. 2014;**39**(7):1322-31. doi: 10.1007/s11064-014-1315-1. [PubMed: 24777807].

20. Kakkar V, Muppu SK, Chopra K, Kaur IP. Curcumin loaded solid lipid nanoparticles: an efficient formulation approach for cerebral ischemic reperfusion injury in rats. *Eur J Pharm Biopharm*. 2013;**85**(3):339-45. doi: 10.1016/j.ejpb.2013.02.005. [PubMed: 23454202].
21. Huang S, Wang B, Zhang Z, Meng Z, Cao H, Lian Q, et al. Effect of curcumin on the expression of high mobility group box 1 and apoptotic neurons in hippocampus after global cerebral ischemia reperfusion in rats. *Zhonghua Yi Xue Za Zhi*. 2011;**91**(19):1340-3. [PubMed: 21756762].
22. Hassanpour A, Hassanpour A, Rezvani ME, Sharifabad MH, Basiri M. The Effect of Intermittent Fasting Diet on the Hippocampus of Adult Male Mouse After Inducing Demyelination by Ethidium Bromide Injection. *Int J Morphol*. 2019;**37**(3):805-14.
23. Baluchnejadmojarad T, Roghani M. Coenzyme q10 ameliorates neurodegeneration, mossy fiber sprouting, and oxidative stress in intrahippocampal kainate model of temporal lobe epilepsy in rat. *J Mol Neurosci.* 2013;**49**(1):194-201. doi: 10.1007/s12031-012-9886-2. [PubMed: 23008120].
24. Amini E, Golpich M, Farjam AS, Kamalidehghan B, Mohamed Z, Ibrahim NM, et al. Brain lipopolysaccharide preconditioning-induced gene reprogramming mediates a tolerance state in electroconvulsive shock model of epilepsy. *Front Pharmacol*. 2018;**9**:416. doi: 10.3389/fphar.2018.00416. [PubMed: 29765321].
25. Faiq MA, Universitas GM, Achmad B, Partadiredja G. Implementation of unbiased stereology method for organ volume estimation using image processing. *EECSI*. 2017;1-6. doi: 10.1109/EECSI.2017.8239093.
26. Altindağ F, Rağbetli MÇ. The effect of maternal treatment with diclofenac sodium and thymoquinone on testicular parameters in rat offspring. *Rev Int Androl*. 2021;**19**(1):34-40. doi: 10.1016/j.androl.2019.07.001. [PubMed: 31628026].
27. Akbari F, Ansari Samani R, Karimi A, Mortazaei S, Shahinfard N, Rafieian-Kopaei M. Effect of turnip on glucose and lipid profiles of alloxan-induced diabetic rats. *Iran J. Endocrinol Metab*. 2013;**14**(5):492-97.
28. Zamani M, Hassanshahi J, Soleimani M, Zamani F, Chubin K. Effects of olive oil consumption on stroke complications, particularly in the hippocampal region, in mice. *Iranian J Nutr Sci Food Technol* . 2012;**7**(2):1-8.
29. Al-Omar FA, Nagi MN, Abdulgadir MM, Al Joni KS, Al-Majed AA. Immediate and delayed treatments with curcumin prevents forebrain ischemia-induced neuronal damage and oxidative insult in the rat hippocampus. *Neurochem Res*. 2006;**31**(5):611-8. doi: 10.1007/s11064-006-9059-1. [PubMed: 16770732].
30. Li W, Suwanwela NC, Patumraj S. Curcumin by down-regulating NF-kB and elevating Nrf2, reduces brain edema and neurological dysfunction after cerebral I/R. *Microvasc Res.* 2016;**106**:117-27. doi: 10.1016/j.mvr.2015.12.008. [PubMed: 26686249].
31. Ahmad N, Umar S, Ashafaq M, Akhtar M, Iqbal Z, Samim M, et al. A comparative study of PNIPAM nanoparticles of curcumin, demethoxycurcumin, and bisdemethoxycurcumin and their effects on oxidative stress markers in experimental stroke. *Protoplasma*. 2013;**250**(6):1327-38. doi: 10.1007/s00709-013-0516-9. [PubMed: 23784381].
32. Jia G, Tan B, Ma J, Zhang L, Jin X, Li C. Prdx6 upregulation by curcumin attenuates ischemic oxidative damage via SP1 in rats after stroke. *BioMed Res Int*. 2017;**2017**: 6597401. doi: 10.1155/2017/6597401. [PubMed: 28596967].
33. Wu J-x, Zhang L-y, Chen YL, Yu SS, Zhao Y, Zhao J. Curcumin pretreatment and post-treatment both improve the antioxidative ability of neurons with oxygen-glucose deprivation. *Neural Regen Res.* 2015;**10**(3):481-9. doi: 10.4103/1673-5374.153700. [PubMed: 25878600].
34. Miao Y, Zhao S, Gao Y, Wang R, Wu Q, Wu H, et al. Curcumin pretreatment attenuates inflammation and mitochondrial dysfunction in experimental stroke: The possible role of Sirt1 signaling. *Brain Res Bull.* 2016;**121**:9-15. doi: 10.1016/j.brainresbull.2015.11.019. [PubMed: 26639783].
35. Liu Z, Ran Y, Huang S, Wen S, Zhang W, Liu X, et al. Curcumin protects against ischemic stroke by titrating microglia/macrophage polarization. *Front Aging Neurosci*. 2017;**9**:233. doi: 10.3389/fnagi.2017.00233. [PubMed: 28785217].
36. Samini F, Samarghandian S, Borji A, Mohammadi G, bakaian M. Curcumin pretreatment attenuates brain lesion size and improves neurological function following traumatic brain injury in the rat. *Pharmacol Biochem Behav*. 2013;**110**:238-44. doi: 10.1016/j.pbb.2013.07.019. [PubMed: 23932920].
37. Xie CJ, Gu AP, Cai J, Wu Y, Chen RC. Curcumin protects neural cells against ischemic injury in N2a cells and mouse brain with ischemic stroke. *Brain Behav*. 2018;**8**(2):00921. doi: 10.1002/brb3.921. [PubMed: 29484272].
38. Tian M, Zhang X, Wang L, Li Y. Curcumin induces ABCA1 expression and apolipoprotein AI-mediated cholesterol transmembrane in the chronic cerebral hypoperfusion aging rats. *Am J Chin Med*. 2013;**41**(05):1027-42. doi: 10.1142/S0192415X13500699. [PubMed: 24117066].
39. Dolatabadi LK, Emamghoreishi M, Namavar MR, Sarkala HB. Curcumin effects on memory impairment and restoration of irregular neuronal distribution in the hippocampal CA1 region after global cerebral ischemia in male rats. *Basic Clin Neurosci*. 2019;**10**(5):527-39. doi: 10.32598/bcn.9.10.365. [PubMed: 32284841].
40. Mukherjee A, Sarkar S, Jana S, Swarnakar S, Das N. Neuro-protective role of nanocapsulated curcumin against cerebral ischemia-reperfusion induced oxidative injury. *Brain Res*. 2019;**1704**:164-73. doi: 10.1016/j.brainres.2018.10.016. [PubMed: 30326199].
41. Ghezzi A, Baroncini D, Zaffaroni M, Comi G. Pediatric versus adult MS: similar or different? *Mult Scler Demyelinating Disord*. 2017;**2**(1):1-14. doi: 10.1186/s40893-017-0022-6.
42. Wu A, Noble EE, Tyagi E, Ying Z, Zhuang Y, Gomez-Pinilla F. Curcumin boosts DHA in the brain: Implications for the prevention of anxiety disorders. *Biochim Biophys Acta*. 2015;**1852**(5):951-61. doi: 10.1016/j.bbadis.2014.12.005. [PubMed: 25550171].
43. Ji B, Han Y, Liu Q, Liu X, Yang F, Zhou R, et al. Curcumin improves the impaired working memory in cerebral ischemia-reperfusion rats by inhibiting proinflammatory cytokines. *Zhonghua Yi Xue Za Zhi*. 2014;**94**(13):1029-33. [PubMed: 24851695].