Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°4, 864-871
DOI: 10.24205/03276716.2020.893

The Effect of Curcumin on Endogenous Neuron Regeneration in Rats after TBI

Yimei Shu[a], Tao Wang[b], Xiaoqiang Tang[b], Changjie Pan[b*]

Abstract

Objective: To investigate the effect of curcumin on endogenous neuron regeneration in rats after traumatic brain injury (TBI) in rats.

Methods: TBI model was prepared with controlled cortical impact model. SD rats were divided into 3 groups: control group, TBI+vehicle group and TBI+curcumin group. Serum SOD and MDA levels were detected by ELISA. The cognitive function of the rats was observed by Morris water maze (MWM). Then the TUNEL method was used to detect the apoptosis of the injured area. NeuN/BrdU immunofluorescence double labeling was used to detect the newly matured neurons in the injured area. Western blot was used to detect DCX protein in the hippocampus.

Results: Curcumin decresed the serum MDA level and increased the serum SOD level in rats with TBI. MWM test showed curcumin decreased the escape latency and increased the number of platform crossover of rats with TBI. The apoptotic cells in the injured area of the rats in the TBI+curcumin group were significantly less than those in other TBI rats. More $NeuN^+/BrdU^+$ double-label positive neonatal mature neurons were observed in the cortex of the TBI+curcumin group, which was more significant than those in other TBI rats. The expression of DCX protein in the hippocampus of the rats in the TBI+curcumin group was dramatically more than that in other TBI rats.

Conclusion: Curcumin can reduce the level of oxidative stress in TBI rats, protect neurons from apoptosis, and promote the development of neurons in the injured cortex and hippocampus, thus improving the learning and memory functions of TBI rats.

Keywords: curcumin, traumatic brain injury, oxidative stress, cognitive function

Introduction

Traumatic brain injury (TBI) is one of the common diseases in clinical brain surgery, and it is caused by external force. According to the World Health Organization (WHO), more than 5 million people worldwide die each year from TBI, which accounts for 9% of all deaths and is 1.7 times the death toll from AIDS, tuberculosis and malaria[1, 2]. More than 1.3 million of these deaths are the result of traffic trauma, and by 2020, it is expected to increase to more than 2 million, which will be one of the leading causes of death and disability[3, 4]. TBI is characterized by the occurrence of neuronal loss, which leads to cognitive function changes and the occurrence of disability. How to protect the neurons from apoptosis and replace the missing neurons has been a hot spot and a difficult point of the research[5, 6].

There is endogenous neurogenesis in the adult central nervous system, and this view has been agreed. It is currently believed that endogenous neurogenesis in the adult central nervous system is mainly in the subventricular zone and the dentate gyrus of the hippocampus[7]. The neurogenesis of the subgranular layer of the hippocampal dentate gyrus is closely related to the learning and memory functions. As the research progressed, it was found that the cortex in the injured area also had neurogenesis. However, in the absence of external intervention factors, the neurogenesis of the injured cortical area is limited. How to improve endogenous neurogenesis to promote the treatment of TBI has become a potential new treatment strategy[7-12].

Curcumin is an active ingredient extracted from the rhizomes of turmeric. Curcumin has a number of pharmacological activities such as improvement

a. The First School of Clinical Medicine, Nanjing Medical University, Nanjing, Jiangsu, China
b. Department of Radiology, Changzhou Second People's Hospital affiliated to Nanjing Medical University, Changzhou, Jiangsu, China
** Author for Correspondence: Changjie Pan*
Department of Radiology, Changzhou Second People's Hospital affiliated to Nanjing Medical University, No. 68 Gehu Middle Road, Changzhou 213164, Jiangsu, China
Email: panchangjie@njmu.edu.cn
Phone/Fax: +86-13815032301

of blood flow, anti-free radicals, and anti-inflammatory effects[13-15]. More and more researches have shown the potential beneficial effects of curcumin in the central nervous system[13, 16, 17]. It has been reported that curcumin had the beneficial effects on cerebral ischemia[14], TBI[18], and Alzheimer's disease[19]. However, the effect of curcumin on the endogenous neuron regeneration in rats after TBI is not clear. This study aimed to investigate the effect of curcumin on the endogenous neuron regeneration in rats after TBI and its possible mechanism, and provide theoretical and experimental basis for the application of curcumin in the treatment of TBI.

Methods

Pets.

The Nanjing Medical University Animal Experimental Center has purchased 36,000-220 g male and female SD rats. In three groups: control group, TBI+vehicle group and the TBI+curcumin group, the random table method was divided into SD rats. Rats were lodged in groups and sex, free to eat and drink, kept in a 12-hour dark / light time (light on from 8:00am to 8:00pm) at a temperature of 25±1 ° C with 65±5% humidity. This study has been approved by the Nanjing Medical University's Ethics Review Committee. The studies have been conducted based on the corresponding guidelines.

Preparation of the TBI model

The rats had been fixed to a stereotatic instrument after abdominal anesthesiology and the cranial tops had been cut and prepared, the skin had been iodine and alcohol disinfested and the skin was scalpel-coated and the fascia separated for the parietal bone exposure. A circlic bone window with a diameter of 5 mm was drilled around the centre by means of a 3.0 mm left side of the sagittal suture and a 3.5 mm posterior crest, which kept the dura mater intact. Rats were subject to controlled cortical impacts using a pneumatic impact device with 1,5 atm (1 Atm=101,325 kPa) (Model FP302, AmScien Instruments LLC, USA). The hematoma appeared immediately in the cortex and the model was prepared successfully. Layer by layer the incision was sutured. Only the bone window has been opened in the control group and the TBI model has not been prepared. During model preparation, there was no accidental death in rats. All rats were injected into the bronchial neuron for 5 days intraperitoneically (50 mg / kg, bid).

Curcumin treatment by tail vein injection

Curcumin (Sigma, US) was injected into rats in TBI+curcumin group at 30 mg / kg immediately after surgery (80 mg curcumin was dissolved in 0.8 ml of absolute ethanol and afterwards 9.2 ml of normal, sterile saline was added in order to produce a solution of curcumin) once per day, for 14 days at the same time. Control and TBI+ group rats were injected by the tail vein with the same quantity of sterile solvent.

Detection of malondialdehyde serum (MDA) and SOD levels SOD

1 ml of blood has been collected from the tail vein of rats at 1, 3, 7 and 14 days of surgery. MDA and SOD levels in the rat serum were detected in MDA and SOD kits (Beyotime Biotechnology, China), and strictly in conformity with kit instructions.

Morris water labyrinth (MWM) observes the cognitive function.

The cognitive functions of rats were observed with the MWM (Huaibeizhenghua, China) at 15 days after surgery, as described by Tian[20]. On the 15th day after the operation, the hidden platform testing was initially performed on the rats, and a recording ended after the rats boarded the platform for 2 s with a max. recording time of 120 s was recorded for the rats looking for hidden platform. Briefly. Rats tested for 4 days three times a day. The platform under the water surface was removed on the 19th day after the operation and testing started to detect the number of crossing rats that cross the platform within 120 s.

TUNEL Fleece

After the MWM test, frozen rat brain slice with a thickness of 15 μm was prepared. The cortical apoptotic cells were detected by the TUNEL kit (Beyotime Biotechnology, Chinese) and strictly followed by the kit instructions. The number of apoptotic cells under a 400-fold field of view was calculated under a fluorescent microscope (Leica DMR).

Figure of immunofluorescence

After MWM test, the rats were prepared with a frozen coronate brain slice of 15 μm thickness. At room temperature, the following primary antibodies were incubated at 6 hours: anti-NeuN mouse (mature marker for the neuron) (1.400, Abcam, UK) and anti-BrdU mouse (1:400, Abcam, UK). The slices were incubated with second-hand antibodies for 2 hours after rinsing with the following: Alexa Fluor ® 568-labeled goat anti-

arbitrary secondary antibody (1:800, Abcam, UK), Alexa Fluor ® 488-labeled goat anti-mouse (1:600, Abcam, UK) with 0,01 mol / L PBS for 3 times. After 3 washings of 0.01 mol / L of PBS, Hoechst 33342 (1:3000) incubated the slices at room temperature for 0.5 h. The number of positive cells was measured using a 400-fold field of view with a fluorescent microscope (Leica DMR, Germany).

Western blot

The fresh hippocampus tissue was taken from the rat after MWM test. The lysate and fully lysed tissue was added to the hippocampal tissue and centribed for 15 minutes at 4 ° C at 12000 g. Each lane has been added 50 μg of the total protein loading buffer. The protein has been transferred to pvdf membrane following SDS / PAGE gel electrophoresis. The mouse anti-β-actin (1:1000, Abcam, UK) were incubated overnight at a temperature of 4 ° C, following blockage, with rabbit anti-DCX (neuronal precursor cell marker) (1:400, Abcam, UK). HRP tagged goat IgG secondary antibody (1:1000, Abcam, UK) was then added and incubated for one hour at room temperature and also for a secondary anti-mouse goat HRP tagged IgG anti-mouse (1:1000, Abcam, UK). Images were taken and the molecular imager ChemiDoc XRS system was used for densitometric analysis.

Analysis of statistics

In order to analyse data in this study, statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 21.0. The data is given as medium ± SD. One-way variance analysis (ANOVA) was used to compare the groups. Statistically significant was considered P<0.05.

Results

The serum MDA level of rats

The TBI+ group of rats' serum MDA levels began to rise 1 day after operation compared to the control group, peaked at 3 days and decreased by 74 days, but still higher. Serum MDA levels of TBi+curcumin have also begun increasing 1 day after the operation to a peak of three days but substantially lower than the TBI+ level of the group and reduced to a normal level of MDA serum (table-1) for seventh or fourteenth days. The concentration of TBI+curcumin also decreased.

The rats' SOD level

In the TBI+ car group, the serum SOD level of rats began to worsen within one day, reached the minimum three-day value, bounced back to 7 and 14 days and remained less compared to the control group. The seral sodium level of TBI+curcumin rats was also reduced at 1 day post-operatively and was lower than TBI+ at 3 days, and the sodium level rates were reduced at 7 and 14 days to the normal level (Table 2). the memory and learning function of rats

In comparison to the TBI+ control groups, the rats were considerably proliferated and the exit latency was also prolonged, but lower than that of TBI+ in the TBI+cumin group (Figure 1A). In comparison to that of TBI+ vehicles and also in the TBI+curcumin group but above that of the TBI+ vehicle group the platform crossover number of rats was significantly reduced (Figure 1B).

Rather apoptosis cortical cells

There were almost no apoptotic cells in the rat cortex in the control group. The cortex was more apoptotic in rats in the TBI+ car group and the TBI + cumin apoptotical cells was significantly smaller in the cortex of the rats than in the TBI+car (Figure-2).

Cortex mature neurons neonatal cortex

There was almost no NeuN+/BrdU+ neonatal mature neurons in the rat cortex within the control group. Many neonatal neurons of NeuN+/BrdU+ were found in the TBI+ curcumin group (Figure 3), which was significantly higher than the TBI+ group..

Hippocampal DCX level of protein

DCX protein expression has decreased significantly in the TBI+ vehicle group rat hippocampus, composed of the control group, and TBI+curcumin expression has decreased, though significantly higher than TBI+ vehicle expression (figure-4).

Discussion

The incidence of TBI is increasing in the clinic. Due to the irreversible cell defect in the injured brain tissue, if it is in an important functional area, most patients will leave limb movement, sensory disturbance or cognitive dysfunction, which seriously affects the life quality of the patients[21]. As well as the society's heavy economic burden, patients and families also suffer tremendous mental stress[21, 22]. How to repair the damaged brain tissue and promote the recovery of patient function has been a problem that has plagued clinicians and researchers, and is also a hot issue at present. This study aimed to investigate the neuroprotective effect of curcumin on TBI in rats.

The TBI model in this study was prepared with the controlled cortical impact using a pneumatic impact device. The injured area of the rat TBI model

was selected in the cortical area above the rat hippocampus, and this area had no effect on the limb motor function of the rat. On the basis of successful modeling, this study first observed the learning and memory of rats. The results of MWM test showed that the learning and memory functions of TBI rats decreased, and the treatment of curcumin could improve the learning and memory functions of TBI rats. What is the possible mechanism of this therapeutic effect of curcumin? Taking this question into consideration, we have observed the antioxidant effect of curcumin. MDA is a lipid oxidation product, which indicates that the level of oxidation in the body. SOD is the main enzyme for antioxidants, and its level represents the antioxidant capacity in the body. The results in this study showed the rats were in oxidative stress after TBI, and curcumin treatment could decrease the serum MDA level and increase the serum SOD level, which indicated curcumin had antioxidant activity. Next, we used the TUNEL kit to observe the apoptosis of the cortex. The result suggested that curcumin could protect the injured cortical cells from apoptosis in TBI rats.

The above results indicated that curcumin had neuroprotective effect on TBI. What is the effect of curcumin on endogenous neurogenesis? Taking this question into consideration, we used NeuN/BrdU immunofluorescence double label to detect the neonatal mature neurons in the injured area. NeuN is one of the mature neuron nuclear antigen markers[23, 24], and BrdU is one of the markers of cell proliferation[25]. The result showed that many $NeuN^+/BrdU^+$ neonatal mature neurons were found in TBI+curcumin group, which was significantly more than that in the TBI+vehicle group, indicating that curcumin promoted neuronal regeneration in the injured cortical area after TBI in rats. In addition, we used Western blot to detect the expression of DCX protein in rat hippocampus. DCX only appears in neuronal precursor cells[26]. The result showed that the expression of DCX protein in the hippocampus of the rats in the TBI+vehicle group was significantly decreased compared with control group, and that in TBI+curcumin group was also reduced, but significantly more than that in the TBI+vehicle group, which indicated the number of newborn neuronal precursor cells in hippocampus after TBI was reduced, after treatment with curcumin, the development of neonatal precursor cells in hippocampus could be significantly promoted.

In summary, curcumin can reduce the level of oxidative stress in TBI rats, protect nerve cells from apoptosis, and promote the regeneration of neurons in the injured cortex and hippocampus, thus improving the learning and memory functions of TBI rats.

Conflict of Interest statement

None.

Acknowledgements

None.

References

[1] Vander Werff Kathy, R., *The Application of the International Classification of Functioning, Disability and Health to Functional Auditory Consequences of Mild Traumatic Brain Injury.* Semin Hear, 2016. **37**(3): p. 216-32.

[2] Chung, P., S.J. Yun, and F. Khan, *A comparison of participation outcome measures and the International Classification of Functioning, Disability and Health Core Sets for traumatic brain injury.* J Rehabil Med, 2014. **46**(2): p. 108-16.

[3] Wofford, K.L., D.J. Loane, and D.K. Cullen, *Acute drivers of neuroinflammation in traumatic brain injury.* Neural Regen Res, 2019. **14**(9): p. 1481-1489.

[4] Ntali, G. and S. Tsagarakis, *Traumatic brain injury induced neuroendocrine changes: acute hormonal changes of anterior pituitary function.* Pituitary, 2019. **22**(3): p. 283-295.

[5] Bao, W., et al., *Complement cascade on severe traumatic brain injury patients at the chronic unconscious stage: implication for pathogenesis.* Expert Rev Mol Diagn, 2018. **18**(8): p. 761-766.

[6] Zhou, Y., et al., *Advance of Stem Cell Treatment for Traumatic Brain Injury.* Front Cell Neurosci, 2019. **13**: p. 301.

[7] Ngwenya, L.B. and S.C. Danzer, *Impact of Traumatic Brain Injury on Neurogenesis.* Front Neurosci, 2018. **12**: p. 1014.

[8] Liao, R., et al., *Histamine H1 Receptors in Neural Stem Cells Are Required for the Promotion of Neurogenesis Conferred by H3 Receptor Antagonism following Traumatic Brain Injury.* Stem Cell Reports, 2019. **12**(3): p. 532-544.

[9] Li, Q., et al., *N-Acetyl Serotonin Protects Neural Progenitor Cells Against Oxidative Stress-Induced Apoptosis and Improves Neurogenesis in Adult Mouse Hippocampus Following Traumatic Brain Injury.* J Mol Neurosci, 2019. **67**(4): p. 574-588.

[10] Yang, Y., et al., *MiR-124 Enriched Exosomes Promoted the M2 Polarization of Microglia and Enhanced Hippocampus Neurogenesis After*

Traumatic Brain Injury by Inhibiting TLR4 Pathway. Neurochem Res, 2019. **44**(4): p. 811-828.

[11] Yan, R., et al., *Hes1 negatively regulates neurogenesis in the adult mouse dentate gyrus following traumatic brain injury.* Exp Ther Med, 2018. **16**(3): p. 2267-2274.

[12] Wu, H., et al., *Growth Differentiation Factor 5 Improves Neurogenesis and Functional Recovery in Adult Mouse Hippocampus Following Traumatic Brain Injury.* Front Neurol, 2018. **9**: p. 592.

[13] Sun, G., et al., *Curcumin alleviates neuroinflammation, enhances hippocampal neurogenesis, and improves spatial memory after traumatic brain injury.* Brain Res Bull, 2020. **162**: p. 84-93.

[14] Rocha-Ferreira, E., et al., *Curcumin: Novel Treatment in Neonatal Hypoxic-Ischemic Brain Injury.* Front Physiol, 2019. **10**: p. 1351.

[15] Wei, G., et al., *Tetrahydrocurcumin Provides Neuroprotection in Experimental Traumatic Brain Injury and the Nrf2 Signaling Pathway as a Potential Mechanism.* Neuroimmunomodulation, 2017. **24**(6): p. 348-355.

[16] Dai, W., et al., *Curcumin provides neuroprotection in model of traumatic brain injury via the Nrf2-ARE signaling pathway.* Brain Res Bull, 2018. **140**: p. 65-71.

[17] Cai, J., et al., *Curcumin mitigates cerebral vasospasm and early brain injury following subarachnoid hemorrhage via inhibiting cerebral inflammation.* Brain Behav, 2017. **7**(9): p. e00790.

[18] Farkhondeh, T., et al., *Impact of curcumin on traumatic brain injury and involved molecular signaling pathways.* Recent Pat Food Nutr Agric, 2019.

[19] Thota, R.N., et al., *Dietary Supplementation with Curcumin Reduce Circulating Levels of Glycogen Synthase Kinase-3beta and Islet Amyloid Polypeptide in Adults with High Risk of Type 2 Diabetes and Alzheimer's Disease.* Nutrients, 2020. **12**(4).

[20] Tian, H., et al., *Analysis of Learning and Memory Ability in an Alzheimer's Disease Mouse Model using the Morris Water Maze.* J Vis Exp, 2019(152).

[21] Fu, T.S., et al., *Health & Economic Burden of Traumatic Brain Injury in the Emergency Department.* Can J Neurol Sci, 2016. **43**(2): p. 238-47.

[22] Kavosi, Z., et al., *The economic burden of traumatic brain injury due to fatal traffic accidents in shahid rajaei trauma hospital, shiraz, iran.* Arch Trauma Res, 2015. **4**(1): p. e22594.

[23] Boekhoorn, K., et al., *The microtubule destabilizing protein stathmin controls the transition from dividing neuronal precursors to postmitotic neurons during adult hippocampal neurogenesis.* Dev Neurobiol, 2014. **74**(12): p. 1226-42.

[24] Yi, X., et al., *Cortical endogenic neural regeneration of adult rat after traumatic brain injury.* PLoS One, 2013. **8**(7): p. e70306.

[25] Taupin, P., *BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation.* Brain Res Rev, 2007. **53**(1): p. 198-214.

[26] Batailler, M., et al., *DCX-expressing cells in the vicinity of the hypothalamic neurogenic niche: a comparative study between mouse, sheep, and human tissues.* J Comp Neurol, 2014. **522**(8): p. 1966-85.

Figure legends

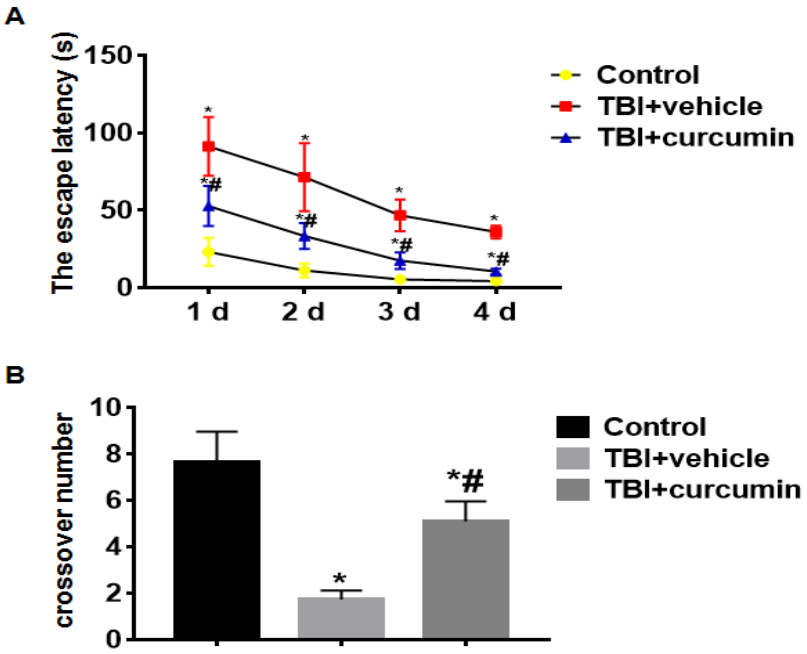

Figure 1. **Morris Water Laze (MWM) has detected the learning and memory functions of rats.**
(A) The escape latency for TBI+ cars was considerably longer compared to the control group and the escape latency of the rats was extended but less than that of the TBI+cars in the TBI+curcumin group. (B) The platform crossover number of rats was substantially decreased compared to the control group in the TBI+ vehicle group and the TBI+curcumin group also decreased, but more than the TBI+ car group. * VS, P<0.05; VS. Vehicle TBI+, P<0.05.

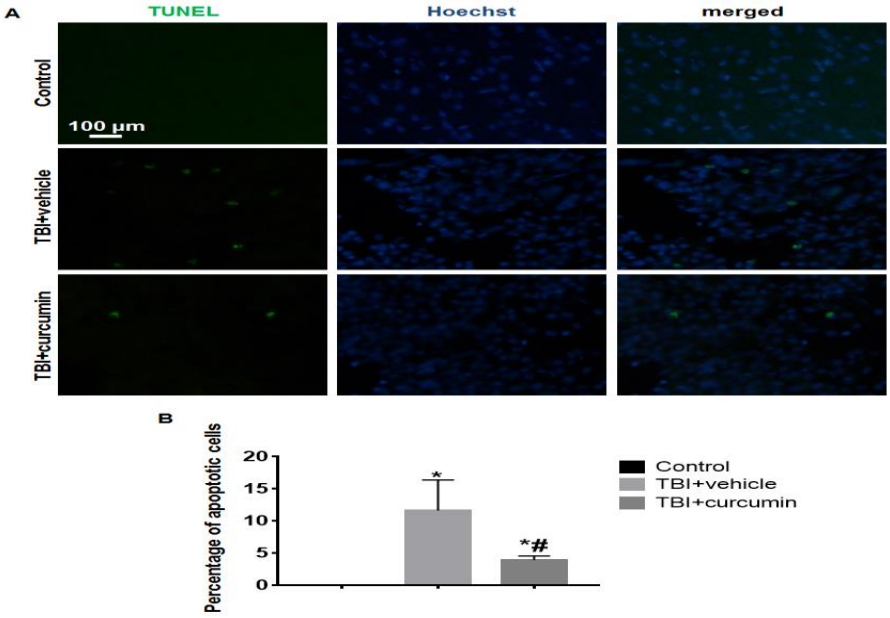

Figure 2. **The TUNEL kit detected apoptotic cells in the cortex.**
(A) In the cortex of rats in the control group almost no apoptotic cells were found. The TBI + group of the rats had more apoptotic cells (white arrow) while the apoptotic cells in the rats' cortex were significantly smaller in the TBI + cumin than in the TBI + car group. (B) Apoptotic cell percentage statistical analytical chart. * Control VS, P<0.05; # VS. Vehicle TBI+, P<0.05. Bar=100μm.

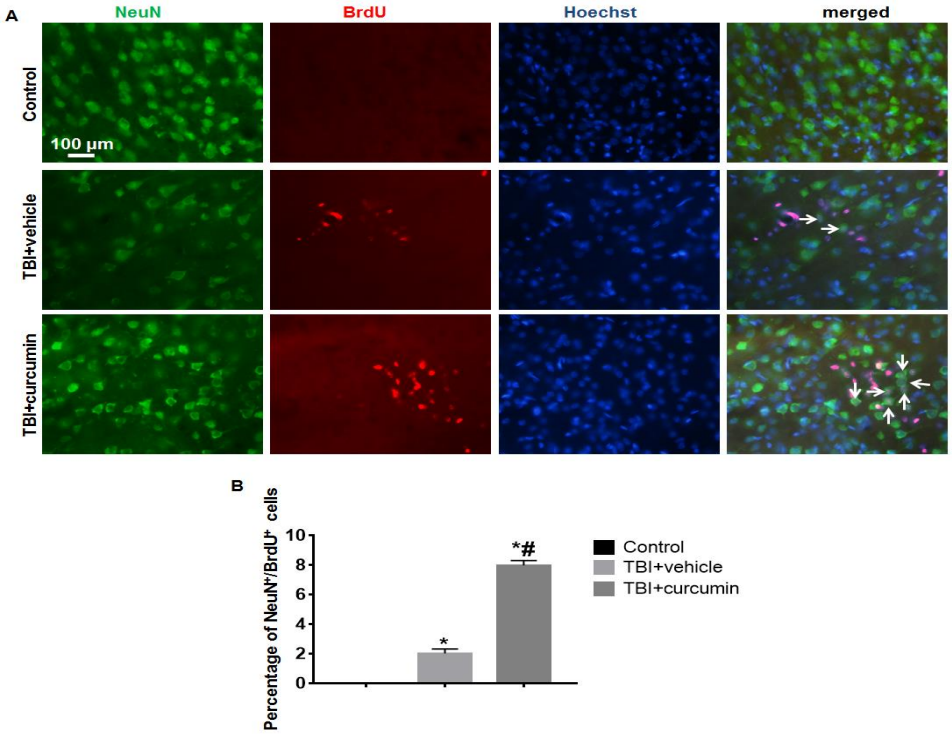

Figure-3. **Immunofluorescence NeuN / BrdU has been detected in mature neurons of the rat cortex.** (A) In the control group of the cortex of rats there were almost no neonatal mature neurons NeuN+/BrdU+. In a group of TBI+curcumin which is considerably higher than in TBI+ vehicles, many NeuN+/BrdU+ neuronal mature neurons (white arrow) were found. (B) NeuN+/BrdU+ neonatal neuronal ripeness graph for statistical analysis. * VS, P<0.05; VS. Vehicle TBI+, P<0.05. Bar=100 microns.

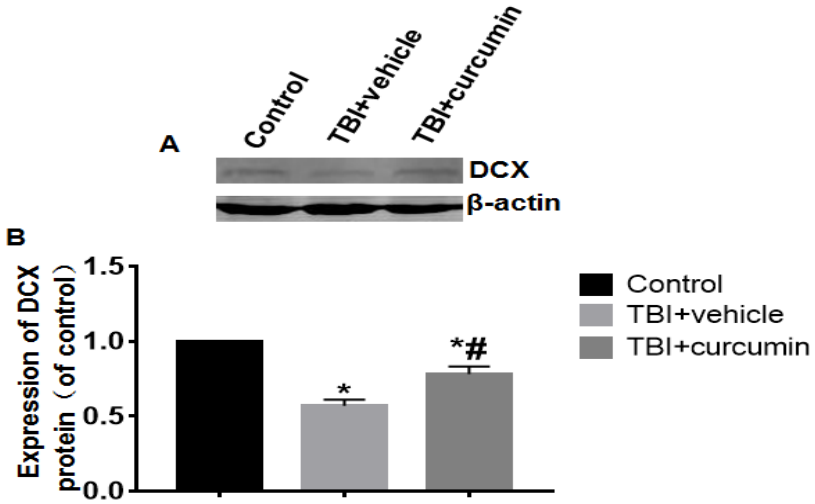

Figure-4. **Western blot was detected in Hippocampal DCX protein expression level.** (A) The expression of the DCX protein in the TBI+ vehicle group in rats has been reduced markedly in comparison to the control group, and the expression has been reduced in the TBI+curcumin group, but significantly above that in the TBI+ vehicles group. (B) Hippocampal DCX protein level statistical analysis graph. * VS, P < 0.05; # VS. Vehicle TBI + P < 05.

Table 1. **The serum MDA level of rats (nmol/mL)**

group	n	1 d	3 d	7 d	14 d
control	6	33.23±4.85	31.75±4.16	30.97±2.89	31.87±5.41
TBI+vehicle	6	54.89±3.35*	103.37±8.58*	76.52±5.18*	46.31±4.32*
TBI+curcumin	6	43.35±6.31*#	73.36±7.10*#	31.46±2.73#	33.14±2.40#
F value		28.35	164.89	288.89	9.26
P value		0.00	0.00	0.00	0.00

Note: * VS. control, $P<0.05$; # VS. TBI+ vehicle, $P<0.05$.

Table 2. **The serum SOD level of rats (nmol/mL)**

group	n	1 d	3 d	7 d	14 d
control	6	151.70±17.69	147.46±9.75	145.87±13.44	155.62±10.36
TBI+vehicle	6	65.52±7.43*	33.60±3.55*	97.14±8.95*	111.61±11.36*
TBI+curcumin	6	93.20±12.34*#	60.36±3.59*#	151.92±17.68#	146.36±14.47#
F value		203.90	103.38	28.33	21.73
P value		0.00	0.00	0.00	0.00

Note: * VS. control, $P<0.05$; # VS. TBI+ vehicle, $P<0.05$.