

Neuroprotective effect of berberine chloride on cognitive impairment and hippocampal damage in experimental model of vascular dementia

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ARTICLE INFO	ABSTRACT
<p>Article type: Original article</p>	<p>Objective(s): The major objective of the present study was to investigate the potential neuroprotective effect of berberine chloride on vascular dementia. Berberine, as an ancient medicine in China and India, is the main active component derived from the <i>Berberis sp.</i> Several studies have revealed the beneficial effects of berberine in various neurodegenerative disorders.</p> <p>Materials and Methods: To induce vascular dementia, chronic bilateral common carotid artery occlusion was performed on male Wistar rats. After surgery, the rats were treated daily by oral administration of berberine chloride (50 mg/kg) for two months. The cognition function of treated rats, were evaluated by Morris Water Maze (MWM) test. In addition, Nissl and TUNEL staining were chosen to assess neuronal damage within the hippocampal CA1 area.</p> <p>Results: It was obvious that chronic cerebral hypoperfusion (CCH), caused cognitive impairment and neuronal damages within CA1 hippocampal subregion. Berberine chloride was able to prevent cognitive deficits, ($P < 0.05$) and reversed CCH-induced hippocampal neuronal loss and apoptosis, ($P < 0.05$).</p> <p>Conclusion: Berberine chloride may be considered as a potential treatment for cognitive deficits and neuronal injury caused by CCH in the hippocampal CA1 area.</p>
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Introduction

Chronic cerebral hypoperfusion (CCH), as a major cause of dementia in elderly people, can lead to vascular dementia (VaD) (1). There are different methods to investigate the underlying mechanism(s) of CCH in an experimental model particularly permanent bilateral common carotid occlusion (2VO) (2).

The obtained results from animal model of CCH show a strong similarity to the patients affected with vascular dementia regarding memory impairments, neuronal degeneration and microvascular abnormalities (3). Moreover, CCH has been reported in cardiovascular disorders including carotid stenosis, occlusion, atherosclerosis and cerebral arterio-venous malformation (4). There are some evidences indicating the role of CCH in neuronal damage to the some area of brain such as hippocampus (5). CCH contributes to the etiology of some neurodegenerative and impaired cognitive disorders like Alzheimer's disease (AD) (1). Previous studies have been shown that

CCH causes almost 60% reduction of cerebral blood flow in the hippocampus (2, 5). The investigation of two important types of cell death including necrosis and apoptosis helps to improve our knowledge regarding neurodegenerative diseases. It has been revealed that 2 weeks after 2VO induction, neural damage was observed and gradually was augmented with time (2). In addition, some studies have indicated that 60 to 90 days after the beginning of occlusion of the vessels, the hippocampus related learning and memory capacity is impaired. The cell death of hippocampus neurons particularly the CA1 pyramidal cell layer, leads to some behavioral changes (6). Berberine (BBR) is an isoquinoline alkaloid which is extracted from the roots and barks of many medicinal plants such as *Berberis*, *Coptidis rhizoma* and *B. integerrima* or *B. vulgaris* with a long history of medicinal applications in India and China (7). Moreover, researchers believe that BBR exerts therapeutic effects such as anti-cancer, anti-microbial, anti-diabetic, anti-

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diarrhea and hypolipidemia has along with anti-oxidative and anti-apoptotic actions (7, 8). Furthermore, there are several studies that show the neuroprotective effects of BBR through *in vivo* or *in vitro* studies in Alzheimer's disease, diabetic neuropathy, Parkinson disease, forebrain ischemia, mental depression and anxiety (9-11). Interestingly, previous studies revealed that BBR has inhibitory effect on neuronal apoptosis in cerebral ischemia due to its high blood brain barrier permeability. On the other hand, BBR protects brain from ischemic injuries by combating against oxidative stress and inhibiting mitochondrial apoptosis (12). Long term administration of BBR is able to ameliorate cognitive impairment in streptozotocin-induced diabetic rats via its anti-apoptotic property (8). Accordingly, it is plausible to consider berberine as a therapeutic agent for the treatment of neurodegenerative disorders including CCH. Based on the aforementioned information, the purpose of the current study was to explore whether berberine can attenuate adverse effects of CCH on learning, memory also neuronal cells death of hippocampus.

Materials and Methods

Animals

Forty male Wistar rats (200-250g) were used in this study. All animals were maintained in a temperature-controlled room ($21\pm 2^{\circ}\text{C}$), on a 12/12 hr light/dark cycle, with food and water available *ad libitum* under standard conventional conditions. All experimental stages were confirmed by Ethics Committee of Yazd University of Medical Sciences which is in accordance with the US National Institutes of Health Publication guide for the care and use of laboratory animals. Rats were randomized and divided into five groups (eight rats per group) including: group I served as an intact group which were not subjected to any intervention, group II sham considered as a control, group III which were subjected to permanent, bilateral common carotid arteries occlusion, group IV was received 50 mg/kg berberine by gavage (orally) once daily (8, 11), group V were subjected to 2VO and received 50 mg/kg berberine. Berberine hydrochloride, (Sigma-Aldrich Co) was dissolved in normal saline.

2VO procedure in rats

The Wistar rats were anesthetized with intra-peritoneal (IP) injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Before anesthetic, atropine sulfate (0.1 mg/kg) was injected intramuscularly to prevent any respiratory distress. After anesthesia and under aseptic surgical techniques, a 2 cm ventral midline skin incision was made in the neck area just above the sternal bone. Following common carotid arteries were then exposed and carefully separated from surrounding muscle and adjacent nerve bundles. by decreasing mortality rates, arterial

occlusion was done with an interval of one week between occlusion of right and left common carotids. The right common carotid artery was first occluded and one week later, the left carotid was occluded as well (13). After every surgery, the incision was stitched and the wound treated with betadine solution. Rectal temperature was maintained at $37.0\pm 0.5^{\circ}\text{C}$ using a heating pad throughout the surgical procedure and after the surgery until recovery from anesthesia.

Morris water maze test (MWM test)

Two months following the 2VO surgery (the last 6 days of the experiment), spatial learning and memory was evaluated using MWM test. The MWM apparatus was consisted a black circular tank (150 cm in diameter, 60 cm in height) which was filled to a depth of 32 cm with water at $26\pm 2^{\circ}\text{C}$. The tank was divided into four quadrants (north, east, south and west) and was surrounded by visual fixed cues. A hidden circular platform (10 cm in diameter) was submerged approximately 2.5 cm below the water surface and was kept in the southeast quadrant throughout the training days. A video camera which was mounted in the ceiling above the center of pool monitored the behavior of the animals consist of latency to escape, distance traveled and time spent in the target quadrant where the platform had been placed during training. Training consisted of one block of 4 trials each day for four consecutive days. Subsequent day probe trial (120sec) was performed in absence of platform to evaluate spatial memory and retrieval capacity. Training was begun by placing animals into the maze to find the hidden platform using four different starting points. Rats were permitted to find the platform in 60 sec and then sit on the platform for 30 sec. Animals which were unable to find the platform in 60 sec were guided to the platform and were permitted sit on it for 30sec. Rats were towel-dried and returned in their home cages after each trial.

Histopathology assessment

Rats were deeply anesthetized after behavioral test 60 days after 2VO surgery. Animals were perfused through the ascending aorta with 200-250 ml saline followed by 200-250ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Following perfusion, the brains were removed and were embedded in the same solution for 48 hr. After dehydration in graded concentration of ethanol, brains were embedded in paraffin blocks for sectioning (14). Coronal serial sections (7 μm thick) were cut at the level of the dorsal hippocampus (4mm posterior from the bregma). Four sections were counted in each brain for histological assay. Histo-logical analysis was done by Nissl and TUNEL staining. Neuronal cell loss was

assessed with Nissl staining by staining of sections with 0.1% Cresylviolet acetate. Intact pyramidal neurons in hippo-campal CA1 subregion were counted under a light microscope using 400X magnification. TUNEL staining was performed using a commercial kit (*In Situ*cell death detection kit, Roche, South San Francisco, CA) according to the manufacturer's protocol. First, the sections were embedded in xylene and ethanol in graded concentration for paraffin removal and dehydration. Tissue sections were subjected to proteolytic pretreatment with proteinase K for 30 min and then slides were rewashed with PBS. Subsequently, to block endogenous peroxidase activity which could lead to false positive results, the sections were incubated in H₂O₂ solution. After washing with PBS in the later step, sections re-incubated in TUNEL Reaction Mixture (5 ml TUNEL-Enzyme solution to 45 ml TUNAL- Label solution) for 1 hr at 37-40 °C in humidified chamber. Then tissue sections were labeled with an anti-fluorescein antibody-conjugated with horseradish peroxidase [Amersham, Piscataway, NJ]. Finally, the tissue sections were incubated with 0.05% 3, 3-diaminobanzidine DAB substrate for 1-2 min to visualize apoptotic cells and counterstained with Gill's hematoxylin for 30 sec. The number of TUNEL-positive cells and total cell number in hippocampus (CA1 region) of brain sections were counted under light microscope with 400X magnification. TUNEL-positive cells were expressed as percentage of total cells (15).

Statistical analysis

Data were presented as Standard error of the mean (mean±SEM). Morris water maze escape latency and traveled distance were analyzed using two-way analysis of variance (ANOVA) with repeated measures followed by the Bonferroni posttest to compare replicate means by row. Other data were analyzed using one way ANOVA followed by Tukey's *post-hoc* test to compare the differences between groups. Statistical analysis was performed using Graph pad prism 5. Differences were considered to be statistically significant between two groups when $P < 0.05$.

Results

The effect of berberine chloride on spatial learning and memory impairment in the 2VO rats

The cognitive function was assessed in the MWM test. The mean escape latency and traveled distance for the 2VO rats increased during the third and fourth session of the four learning sessions compared to sham group (Figures 1, 2). In the first and second sessions of escape latency trial, there weren't significant difference between the groups. 2VO animals had a significant impairment in escape latency during the third and fourth sessions (42.33 and 44.67) as compared to sham animals (25 and 26.21) ($P < 0.01$). This increase in latency was attenuated significantly by berberine treatment in the fourth session in the 2VO

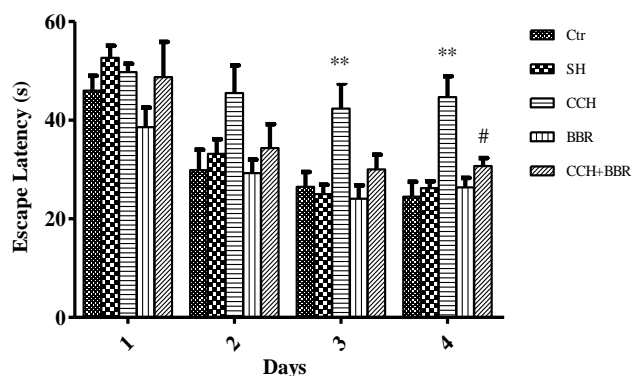


Figure 1. Escape latency in morris water maze test (mean±SE, n=8) in the different groups. Statistical analysis was performed using two-way analysis of variance (ANOVA) with repeated measures followed by the bonferroni post test. ** $P < 0.01$ vs. SH group. # $P < 0.05$ vs. CCH group (Ctr= Control, SH=Sham, CCH= Chronic Cerebral Hypoperfusion, BBR= Berberine)

group (30.71, $P < 0.05$) (Figure 1). Figure 2 shows that the mean traveled distance for the 2VO rats was increased during the third and fourth session of the four learning sessions (552.2 and 493.4) compared to sham group (304.9 and 278, $P < 0.05$). A significant reduction in distance traveled was observed when berberine was intragastrically administrated in 2VO group (243.8, $P < 0.05$).

The result of the probe trial (Figure 3) indicated that rats in the 2VO group spent significantly less time in the target quadrant (10.79) compared to sham group (22.67, $P < 0.05$). A significant increase in spent time in the target quadrant was observed when berberine was intragastrically administrated in the 2VO group (20.5, $P < 0.05$).

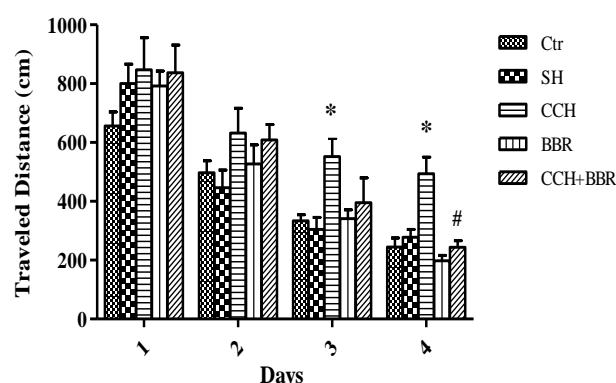


Figure 2. Traveled distance in Morris water maze test (mean±SE, n=8) in the different groups. Statistical analysis was performed using two-way analysis of variance (ANOVA) with repeated measures followed by the bonferroni post test.* $P < 0.05$ vs. SH group. # $P < 0.05$ vs. CCH group. (Ctr= Control, SH=Sham, CCH= Chronic Cerebral Hypoperfusion, BBR= Berberine)

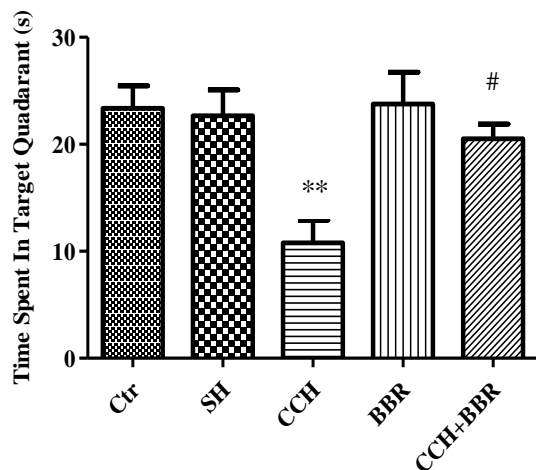


Figure 3. Time spent in target quadratant in Morris water maze test (mean±SE, n=8) in the different groups. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the tukeypost test. ** $P<0.01$ vs. sham SH group. # $P<0.05$ vs. CCH group (Ctr= Control, SH=Sham, CCH= Chronic Cerebral Hypoperfusion, BBR= Berberine)

The effect of berberine chloride on histological changes in the 2VO rats

TUNEL and Nissl staining for the hippocampal CA1 subfield were carried out to investigate the mechanism underlying the degradation of the neurons. Apoptotic index (the percentage of apoptotic to total cells) were significantly increased in the hippocampal CA1 subfield in the 2VO group (42.27%) compared with sham group (1.57%, $P<0.001$), whereas berberine treatment significantly decreased the apoptotic index in CA1 region in group which experienced CCH surgery (24.37%, $P<0.05$) also this group showed a significantly elevation in TUNEL positive cells compared with sham group (1.57, $P<0.05$) (Figures 4 and 5).

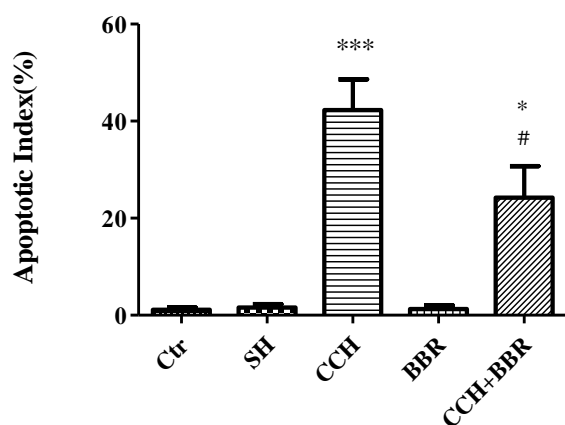


Figure 5. The percentage of apoptotic to total cells (Apoptotic index) (mean±SE, n=4) in the hippocampal CA1 subfield in the different groups. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the tukey post test. *** $P<0.001$ and * $P<0.05$ vs. SH group. # $P<0.05$ vs. CCH group. (Ctr= Control, SH=Sham, CCH= Chronic Cerebral Hypoperfusion, BBR= Berberine)

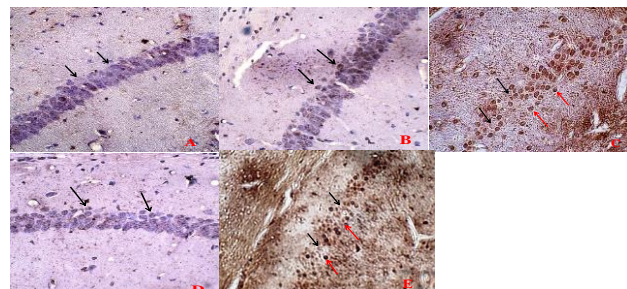


Figure 4. TUNEL staining of hippocampal CA1 region. A: control group (Ctr), B: Sham (SH) group, C: Chronic Cerebral Hypoperfusion (CCH) group, D: Berberine (BBR) group and E: CCH + BBR group. Black arrows indicate intact cells and red arrows indicate apoptotic cells (magnification ×400)

Data from Nissl staining showed that permanent bilateral common carotid occlusion that caused marked CA1 cell loss in the 2VO rats (54.8) versus sham group (120, $P<0.01$). Berberine treatment significantly increased cell density in the hypo-perfused animals (105.6, $P<0.05$, Figures 6, 7).

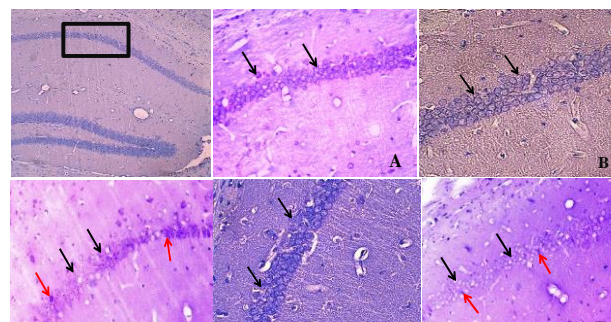


Figure 6. Nissl staining of hippocampal CA1 region. A: control group (Ctr), B: Sham (SH) group, C: Chronic Cerebral Hypoperfusion (CCH) group, D: Berberine (BBR) group, E: CCH+BBR group. Black arrows indicate intact cells and red arrows indicate necrotic cells (magnification ×400)

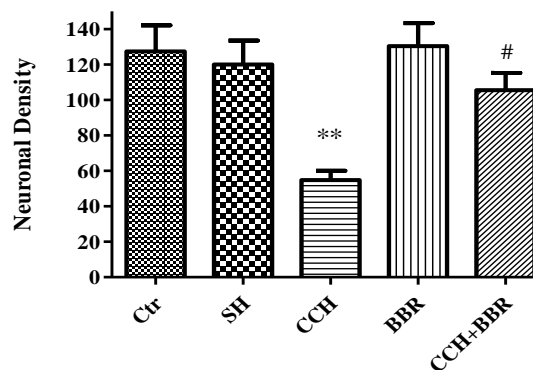


Figure 7. Neuronal density (mean±SE, n=4) in the hippocampal CA1 subfield in the different groups. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the tukey post test. ** $P<0.01$ vs. SH group. # $P<0.05$ vs. CCH group (Ctr= Control, SH=Sham, CCH= Chronic Cerebral Hypoperfusion, BBR= Berberine)

Discussion

In the present study, we examined the beneficial effect of berberine chloride on cognitive impairment and hippocampus tissue damage in an experimental model of chronic cerebral hypoperfusion. For this purpose, permanent bilateral common carotid occlusion was performed in selected rats to evaluate the behavioral and histological alternations in the animals. The major findings obtained from the present study are as following: (1) CCH induced a decrease in spatial learning and memory; (2) Berberine administration to the rats prevented CCH-induced diminution in spatial learning and memory; (3) CCH led to histological injuries in hippocampus while berberine prevented histological changes in the rats under CCH induction. Of note, the results of the present study are in accordance with the previous results which showed that induction of CCH led to a cognition dysfunction and tissue damages two months after the onset of the model (13). In addition, the present study provided evidence in favor of the beneficial effect of berberine administration (50 mg/kg) on decreasing the cognition dysfunction of rats such as escape latency, traveled distance also increasing the time spent in target quadranant in the rat model of CCH which was assayed by MWM test.

Intellectual abilities like learning and memory can be affected by central cholinergic functions. Similar to the pathological changes which are observed in AD and VaD patients usually show cholinergic abnormalities and serious cognitive disorders (16).

The acetyl cholinesterase (AChE) concentration in the cerebrospinal fluid of VaD patients has been shown to be significantly lower than and to be significantly linked with dementia scale scores (17). In addition, post-mortem studies have revealed that the brain activity of choline acetyl transferase (ChAT) in affected individuals was decreased in different parts of CNS like cortex, hippocampus, and striatum (18). Berberine is able to inhibit AChE activity and also protect cholinergic neurons in the hippocampus. Moreover, berberine boosts the enzymatic ChAT and AChE activities, which in turn leads to improving the function of entire cholinergic circulation pathway (19, 20). Based on the presented information, cholinesterase inhibitors on one hand, and ChAT activators on the other hand, are able to exert compensatory effect for increasing the ACh levels in brains of patients with AD disease.

Furthermore, the present study has explored the efficacy of administration of berberine to the CCH rat models. Interestingly, a significant decrease in both types of cell death (apoptosis and necrosis) was observed in CA1 hippocampus neurons of CCH rats treated with beneficial dose of berberine.

The underlying mechanism by which this action takes place is still unknown, but considering the

findings of previous studies concerning the neuronal death induced by free radical formation, brain inflammatory response (21, 22). It seems any factors lead to inhibit this process can be used in treatment of vascular dementia. It seems that berberine exerts its neuroprotective effects at least in part through attenuation of oxidative stress.

Chronic cerebral hypoperfusion leads to oxidative stress which in turn causes generation of reactive oxygen species and reactive nitrogen species (3). This phenomenon causes the brain energy failure in neuronal tissues and cells. Protein oxidation and DNA damage are important examples in which increased concentration of reactive oxygen and nitrogen species can induce neuronal death (23). Berberine acts against oxidative stress through induction the enzymatic activity of anti-oxidative enzymes superoxide dismutase (SOD) and glutathione (GSH) (24, 25). In addition CCH increased malondialdehyde (MDA), a marker of lipid peroxidation, and nitrite levels in the hippocampus region of the brain (26) and also berberine decrease MDA, and exerts neuroprotective effect (27).

The other possible mechanism for neuroprotective ability of berberine could be related to its capacity in anti-inflammatory effects.

Pro inflammatory pathways which are activated due to chronic cerebral hypo-perfusion is characterized by production oftumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) (28). Therefore, inhibition of these inflammatory mediators is a good candidate for alleviate the brain injury induced by chronic cerebral hypoperfusion. Previous studies showed that berberine protected the neurons by suppression the production of TNF- α , cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (29, 30).

Conclusion

These results indicate that administration of berberine at a dose of 50 mg/kg could have protective effect in hippocampus against CCH and this component may be suggested along with other available therapies in vascular dementia.

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References

1. Lee JS, Im DS, An Y-S, Hong JM, Gwag BJ, Joo IS. Chronic cerebral hypoperfusion in a mouse model of Alzheimer's disease: an additional contributing factor of cognitive impairment. *Neurosci Lett* 2011;489:84-88.
2. Farkas E, Luiten PG, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* 2007;54:162-180.

3. Liu H, Zhang J. Cerebral hypoperfusion and cognitive impairment: the pathogenic role of vascular oxidative stress. *Int J Neurosci* 2012;122:494-499.
4. Pereira FM, Ferreira EDF, de Oliveira RMW, Milani H. Time-course of neurodegeneration and memory impairment following the 4-vessel occlusion/internal carotid artery model of chronic cerebral hypoperfusion in middle-aged rats. *Behav Brain Res* 2012;229:340-348.
5. Jian H, Yi-Fang W, Qi L, Xiao-Song H, Gui-Yun Z. Cerebral blood flow and metabolic changes in hippocampal regions of a modified rat model with chronic cerebral hypoperfusion. *Acta Neurol Belg* 2013; 113:313-317.
6. Shamsaei N, Khaksari M, Erfani S, Rajabi H, Aboutaleb N. Exercise preconditioning exhibits neuroprotective effects on hippocampal CA1 neuronal damage after cerebral ischemia. *Neural Regen Res* 2015; 10:1245-50.
7. Ye M, Fu S, Pi R, He F. Neuropharmacological and pharmacokinetic properties of berberine: a review of recent research. *J Pharm Pharmacol* 2009;61:831-837.
8. Moghaddam HK, Baluchnejadmojarad T, Roghani M, Khaksari M, Norouzi P, Ahoie M, *et al.* Berberine ameliorate oxidative stress and astrogliosis in the hippocampus of STZ-induced diabetic rats. *Mol Neurobiol* 2014;49:820-826.
9. Ji H-F, Shen L. Berberine: a potential multipotent natural product to combat Alzheimer's disease. *Molecules* 2011;16:6732-6740.
10. Kim SO, Kim HJ. Berberine ameliorates cold and mechanical allodynia in a rat model of diabetic neuropathy. *J Med Food* 2013;16:511-517.
11. Kim M, Cho K-H, Shin M-S, Lee J-M, Cho H-S, Kim C-J, *et al.* Berberine prevents nigrostriatal dopaminergic neuronal loss and suppresses hippocampal apoptosis in mice with Parkinson's disease. *Int J Mol Med* 2014;33:870-878.
12. Pires ENS, Frozza RL, Hoppe JB, de Melo Menezes B, Salbego CG. Berberine was neuroprotective against an in vitro model of brain ischemia: survival and apoptosis pathways involved. *Brain Res* 2014;1557:26-33.
13. Cechetti F, Worm PV, Pereira L, Siqueira IR, A Netto C. The modified 2VO ischemia protocol causes cognitive impairment similar to that induced by the standard method, but with a better survival rate. *Braz J Med Biol Res* 2010;43:1178-1183.
14. Mehrjerdi FZ, Aboutaleb N, Pazoki-Toroudi H, Soleimani M, Ajami M, Khaksari M, *et al.* The protective effect of remote renal preconditioning against hippocampal ischemia reperfusion injury: role of kATP channels. *J Mol Neurosci* 2015;57:554-560.
15. Khaksari M, Mehrjerdi FZ, Rezvani ME, Safari F, Mirgalili A, Niknazar S. The role of erythropoietin in remote renal preconditioning on hippocampus ischemia/reperfusion injury. *J Physiol Sci* 2016:1-9.
16. Ni J-W, Matsumoto K, Li H-B, Murakami Y, Watanabe H. Neuronal damage and decrease of central acetylcholine level following permanent occlusion of bilateral common carotid arteries in rat. *Brain Res* 1995;673:290-296.
17. Román GC. Cholinergic dysfunction in vascular dementia. *Curr Psychiatry Rep* 2005;7:18-26.
18. Giacobini E. Cholinergic receptors in human brain: effects of aging and Alzheimer disease. *J Neurosci Res* 1990;27:548-560.
19. Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P, *et al.* Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. *Behav Brain Res* 2011; 220:30-41.
20. Huang L, Su T, Shan W, Luo Z, Sun Y, He F, *et al.* Inhibition of cholinesterase activity and amyloid aggregation by berberine-phenyl-benzoheterocyclic and tacrine-phenyl-benzoheterocyclic hybrids. *Bioorganic Med Chem* 2012;20:3038-3048.
21. Oh SM, Betz AL. Interaction between free radicals and excitatory amino acids in the formation of ischemic brain edema in rats. *Stroke* 1991;22:915-921.
22. Matsuo Y, Kihara T, Ikeda M, Ninomiya M, Onodera H, Kogure K. Role of neutrophils in radical production during ischemia and reperfusion of the rat brain: effect of neutrophil depletion on extracellular ascorbyl radical formation. *J Cereb Blood Flow Metab* 1995;15:941-947.
23. Erfani S, Aboutaleb N, Oryan S, Shamsaei N, Khaksari M, Kalalian-Moghaddam H, *et al.* Visfatin inhibits apoptosis and necrosis of hippocampus CA3 cells following transient global ischemia/reperfusion in rats. *Int J Pept Res Ther* 2015;21:223-228.
24. Hur JM, Hyun MS, Lim SY, Lee WY, Kim D. The combination of berberine and irradiation enhances anti-cancer effects via activation of p38 MAPK pathway and ROS generation in human hepatoma cells. *J Cell Biochem* 2009;107:955-964.
25. Ahmed T, Abdollahi M, Daglia M, Nabavi SF, Nabavi SM. Berberine and neurodegeneration: A review of literature. *Pharmacol Rep* 2015;67:970-979.
26. Saxena AK, Majeed A, Saad S, Oothuman P, Mahmoud M, Al-Deen MI. Lipid peroxidation in chronic cerebral hypoperfusion-induced neurodegeneration in rats. *Inter Med J Malaysia* 2011;10:3-6.
27. Zhang J, Yang J-Q, He B-C, Zhou Q-X, Yu H-R, Tang Y, *et al.* Berberine and total base from rhizoma coptis chinensis attenuate brain injury in an aluminum-induced rat model of neurodegenerative disease. *Saudi Med J* 2009;30:760-766.
28. Tomimoto H, Ihara M, Wakita H, Ohtani R, Lin J-X, Akiguchi I, *et al.* Chronic cerebral hypoperfusion induces white matter lesions and loss of oligodendroglia with DNA fragmentation in the rat. *Acta Neuropathol* 2003;106:527-534.
29. Kim S, Choi JH, Kim JB, Nam SJ, Yang J-H, Kim J-H, *et al.* Berberine suppresses TNF- α -induced MMP-9 and cell invasion through inhibition of AP-1 activity in MDA-MB-231 human breast cancer cells. *Molecules* 2008;13:2975-2985.
30. Domitrović R, Cvijanović O, Pernjak-Pugel E, Škoda M, Mikelić L, Crnčević-Orlić Ž. Berberine exerts nephroprotective effect against cisplatin-induced kidney damage through inhibition of oxidative/nitrosative stress, inflammation, autophagy and apoptosis. *Food Chem Toxicol* 2013;62:397-406.