Journal of Pharmaceutical and Health Sciences 2014;2(2), 85-92.

Original Article

Effect of Coenzyme Q10 (ubiquinone) on hippocampal CA1 pyramidal cells following transient global ischemia/reperfusion in male wistar rat

Open Access

Elham Hashemzadeh[1], Shabnam Movassaghi[2], Hamed Shafaroodi[1], Alieh Ashoori Barmchi[1], Zahra Nadia Sharifi[2*]

[1] *Department of Pharmacology & Toxicology, Pharmaceutical Science Branch, Islamic Azad University, Tehran, Iran.*

[2] *Department of Anatomy, Tehran Medical Branch, Islamic Azad University, Tehran, Iran.*

Abstract

Ischemia/Reperfusion (I/R)-induced cerebral injury has been reported as a leading cause of death and long-term disabilities. Hippocampus is an area which is more sensitive to be affected by I/R and hypoxic conditions. Coenzyme Q10 is a strong antioxidant which plays a role in membrane stabilization. This study aims to investigate the possible role of CoQ10 in ameliorating the histomorphological changes in the hippocampal CA[1] pyramidal cells induced by cerebral I/R.

Thirty six adult male wistar rats were divided into six groups, each group consisting of six rats, including control, ischemia, vehicle and CoQ10-treated (10, 50,100 mg/kg). In treatment groups, rats were orally administered CoQ10 during five days before and three days after I/R. Global cerebral ischemia was induced by bilateral common carotid arteries occlusion for about 20 minutes, followed by reperfusion. H&E and Nissl staining were utilized for some qualitative and quantitative studies. Then the histomorphological changes were measured by Image Tools 2 software.

The data analysis showed a significant reduction in the number of CA[1] pyramidal cells after I/R; whearas, no significant difference was seen in the number of cells in mentioned region between control and 100 mg/kg CoQ10-treated groups.

The findings indicate the neurotrophic properties of CoQ10 and support the beneficial effects of CoQ10 as an adjuvant therapy in patients who have risk factor(s) of ischemic stroke.

Keywords: Hippocampus, Ischemia, Reperfusion, Coenzyme Q10, Rat

***Corresponding author**: *Zahra Nadia Sharifi, Department of Anatomy, School of Medicine, Islamic Azad University-Tehran Medical Branch, Tehran, Iran.*
Tel.:+98-22006660-7 internal 257
Email address: *nadiasharifi@yahoo.com*

Introduction

Normal brain function is highly dependent on adequate blood flow and substrate delivery for production of adenosine triphosphate (ATP) in mitochondria (Aliev et al., 2004; Aliev et al., 2009a). Ischemia/Reperfusion (I/R)-induced cerebral injury has been reported as a leading cause of death and long-term disabilities with consequent impact on the quality of life (Harukuni and Bhardwaj, 2006). During ischemia, the interruption of blood supply and the lack of oxygen in the brain cause inhibition of aerobic glucose metabolism, production and accumulation of lactate which induces tissue acidosis and cellular damage (Siesjö et al., 1996). Cytosolic Ca^{2+} is also elevated during global ischemia and reperfusion (Nakamura et al., 1999). Ca^{2+} influx occurs via opening of voltage-dependent Ca^{2+} channels as a result of drop in adenosine triphosphate (ATP) levels and membrane depolarization (Schurr and Rigor 1992). Other mechanisms resulting in Ca^{2+} overload include glutamate-induced N-methyl D-Aspartate (NMDA) receptor hyperactivation (Rothman and Olney 1986), reversal of the Na^{+}-Ca^{2+} exchanger (Stys et al., 1991) and inhibition of the Ca^{2+}-ATPase (Lipton and Lobner, 1990). Reduction of brain GSH level is the other consequence of I/R (Yousuf et al., 2007). Depletion of brain GSH was attributed to conjugation of GSH to lipid-derived oxidation products, or perturbation in its synthesis process due to energetic impairment (Brongholi et al., 2006). In another word, among the factors involved in the pathogenesis of I/R injury are energy failure, lactic acidosis, glutamate receptor-mediated excitotoxicity, enhanced formation of free radicals and cellular calcium homeostasis disturbance (Harukuni and Bhardwaj, 2006).

However injury is known to occur much more at the onset of reperfusion following cerebral ischemia (Mori et al., 1999). Nevertheless, resultant oxidative burden is the unavoidable byproduct of oxygen-based (aerobic) respiration and hypoperfusion injury (Aliev et al., 2009a). During reperfusion, the stimulated metabolism of hypoxanthin via the xanthine oxidase pathway and the oxidative metabolism of the membrane arachidonic acid cause a burst of reactive oxygen species (ROS) (Khalil et al., 2006). In addition, lactic acidosis was reported to enhance ROS formation via releasing iron from its protein bound forms (Siesjö et al., 1996). In that regard, many antioxidants are reported to reduce ROS-mediated damage in animal models of cerebral ischemia (Aliev et al., 2009a).

Brain is more vulnerable to reactive oxygen species (ROS) induced damage due to its high rate of oxygen consumption, high polyunsaturated lipid content and relative lack of classic antioxidant enzymes (Aliev et al., 2008). Hippocampus which is localized in temporal lob of the brain and plays an important role in transferring immediate or short term memories into long term memories, is an area which is more sensitive to I/R and hypoxic conditions in comparison with other parts of the brain (Robertson, 2002; Smith, 2003).

Ubiquinone an endogenous quinine that is vital for optimum biological function (Dhanasekaran and Ren 2005) is a strong antioxidant influencing membrane stabilization (Folkers et al., 1990). Primary CoQ10 deficiency is an autosomal recessive condition with a clinical spectrum that encompasses at least five major phenotypes, including (a) encephalomyopathy characterized by the trial of recurrent myoglobinuria, brain involvement and ragged red fibers; (b) sever infantile multisystemic disease; (c) cerebellar ataxia; (d) Leigh syndrome with growth retardation, ataxia and deafness; and (e) isolated myopathy (Quinzii et al., 2007). Altered levels of CoQ10 have been reported to be involved in neurodegenerative disorders, cancer and cardiovascular diseases, indicating its involvement in the cellular mechanisms of these ailments (Dhanasekaran and Ren, 2005).

This study aimed to investigate the possible role of ubiquinone in ameliorating the histomorphological changes in the hippocampal CA^{1}

pyramidal cells induced by cerebral I/R in male wistar rats. This study is being carried out in order to explore the probable beneficial effects of the use of ubiquinone as an adjuvant therapy in patients with the risk of ischemic stroke.

Materials and Methods

Animals and materials

Thirty six male wistar rats weighing 250-300 g were used in this study. Animals were housed under controlled conditions and allowed free access to water and standard chow diet ad libitum. All procedures used in this study were approved by the ethics committee of National Institutes of Health (NIH). CoQ10, was purchased from Sigma-Aldrich (USA), dissolved in soybean oil as its solvent.

Surgical procedure

After 5 days from the beginning of the experiment, animals were anesthetized by sodium pentobarbital (40 mg/kg, IP). A rectal temperature probe was inserted and body temperature was monitored and maintained at approximately 37.0 °C with a heating pad.

Briefly, both common carotid arteries were surgically exposed and occluded by microsurgery clamps for about 20 minutes, followed by declamping of both carotid arteries to allow reperfusion. Then rats were sacrificed after 9 days by decapacitation after perfusion intracardiacally. Brains were rapidly removed and put in the fixator for more than 3 days.

Experimental groups

Animals were randomly divided into six experimental groups each consisting of of six rats as described below:

1- Control group: rats were only anesthetized by pentobarbital sodium (40 mg/kg).

2- Ischemia group: After anesthesia with pentobarbital sodium, common carotid arteries on both sides were occluded for 20 min followed by reperfusion.

3- Vehicle group: rats were orally administered soybean oil during 5 days before and 3 days after induction of cerebral I/R.

4- Treatment group 1: Rats were orally administered CoQ10 (10 mg/kg) during 5 days before and 3 days after induction of cerebral I/R.

5- Treatment group 2: Rats were orally administered CoQ10 (50 mg/kg) during 5 days before and 3 days after induction of I/R.

6- Treatment group 3: Rats were orally administered CoQ10 (100 mg/kg) during 5 days before and 3 days after induction of I/R.

Histomorphological studies

At the end of the experiments, the animals were sacrificed and brains were harvested for morphometric studies. Slices (5μm thick) were stained with hematoxylin and eosin (H&E) for qualitative evaluation. For Nissl staining, 10 μm-thick sections were mounted directly on gelatin-coated glass slides and air-dried in order to do quantitative studies. Images were taken at ×40 magnification with a microscope (Olympus AX-70). The number of normal hippocampal CA^1 pyramidal cells in stained sections (3 sections of the hippocampus of each rat between the levels of 2.3 and 5 mm posterior to bregma fortune) were counted by blindly investigation. The diameter of cells was also measured. Image Tools Version 2 software was used in order to investigate CA^1 pyramidal cells.

Statistical Analysis

The SPSS statistical software package was used to do statistical analysis. Statistical significance of differences was evaluated by means of one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison. Statistical significance was defined as a P value = 0.05.

Results

Data from H&E staining showed extensive

neuronal changes in the CA1 regions of the hippocampi in ischemic brains. More shrunken neurons with pycnotic nuclei were found in ischemia and vehicle groups, compared with the other groups.

Data from Nissl staining showed a significant reduction in the number of surviving neurons with round and palely stained nuclei in the CA1 region after cerebral I/R. No significant difference was seen in the number of cells in mentioned region between control and 100 mg/kg CoQ10-treated groups (P=0.063) (Figures 1, 2). The average number of surviving cells in the hippocampal CA1 region of 100 mg/kg CoQ10-treated ischemic rats was significantly higher than other treated groups (n=6). The mentioned dose of CoQ10 administration could also increase the diameter of cells and a significant difference was observed between ischemia group and 100 mg/kg CoQ10-treated group (Figure3).

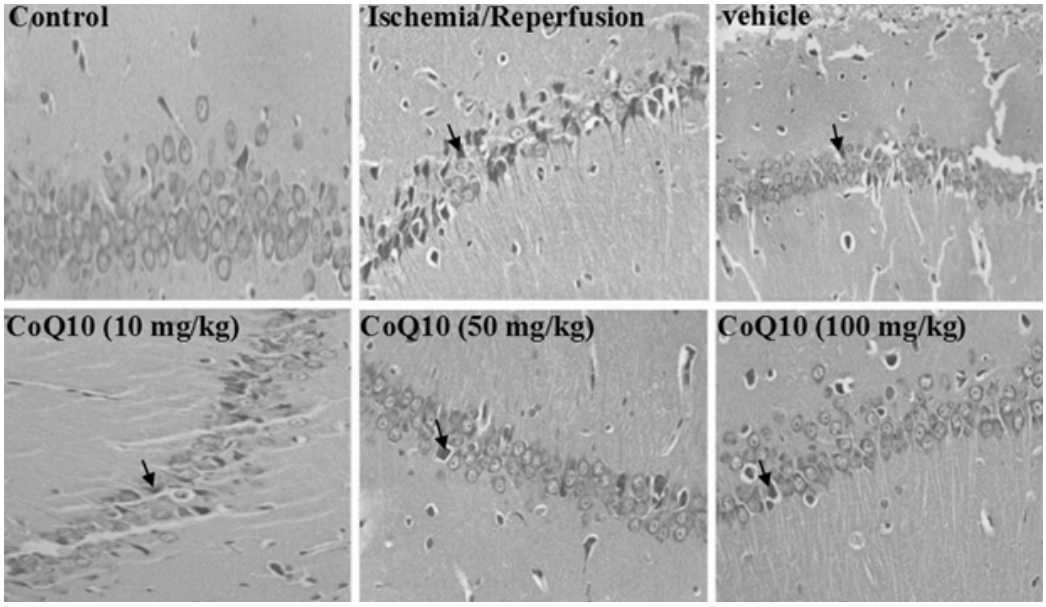

Figure1: Photomicrographs of coronal sections of CA[1] region of hippocampus (Nissl staining, ×40). Arrows indicate the dark shrunken damaged neurons.

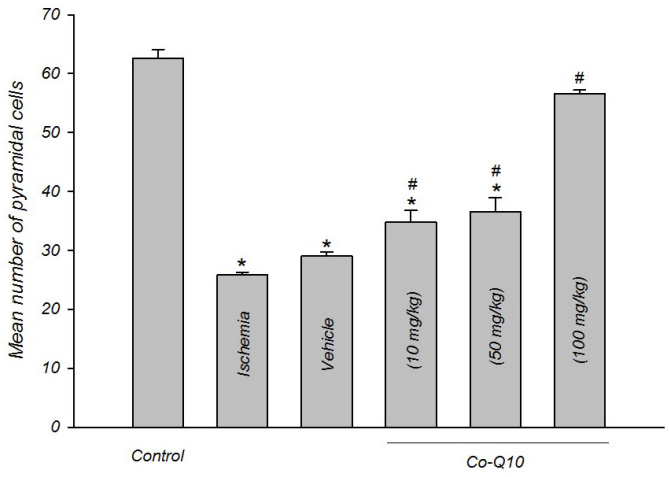

Figure2: Effect of different doses of CoQ10 on number of normal hippocampal CA[1] pyramidal cells in animal models of cerebral ischemia/reperfusion. *= Significantly different as compared to control group at p>0.001.

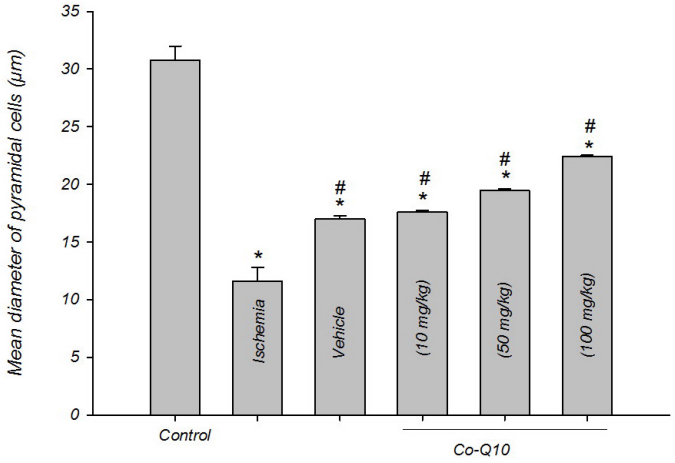

Figure3: Comparison of mean diameter of cells between groups. *= significantly different as compared to control group at p>0.001. #= Significantly different as compared to ischemia group at p>0.01.

Discussion

The above-mentioned findings suggest the potential usefulness of CoQ10 as a neurotrophic supplement to minimize the detrimental histomorphologic changes caused by cerebral I/R. According to the observations: The more CoQ10 consumption, the more neurotrophic effect on CA1 hippocampal cells following I/R. Low intracellular oxygen level is considered as a result of cerebral ischemia/reperfusion and leads to cell metabolism impairment followed by cell death (Hammerman et al., 2002; Wu et al., 2004). An inflammatory response involving the expression of adhesion molecules and cytokines has been observed due to ischemia/reperfusion (Sari et al., 2013).

In addition, hippocampal CA1 pyramidal cells are more sensitive to hypoxia in comparison with other neurons in brain. Free radicals accumulation around these neurons causes necrosis and cell apoptosis (Orrenius et al., 2007; Simone et al., 2007).

Coenzyme Q10 (ubiquinone), located in the mitochondria, lysosomes and Golgi membrane, is fundamental for normal function of these cellular organelles, particularly in mitochondria (Pourahmad and Hosseini 2012). It is known to provide an effective antioxidant protection by reacting directly with free radicals or by being involved in the regeneration of oxidized tocopherol and ascorbate (Aliev et al., 2009b; Obrenovich et al., 2010; Vančová et al., 2010; E Obrenovich et al., 2011).

Previous studies suggest that CoQ10 deficiency may be associated with Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Friedreich's ataxia and other conditions which have been linked to mitochondrial dysfunction. These studies, hence, support the usefulness of CoQ10 in the treatment of neurodegenerative diseases (Mancuso et al., 2010). On the other hand, defects in ATP production and increased reactive oxygen species (ROS) formation, which have been demonstrated during cerebral ischemia (Chan 2001), can induce mitochondria-dependent cell death, as damaged mitochondria cannot maintain the energy demands of the cell (Aliev et al., 2009a). It has also been demonstrated that CoQ10 ameliorates most of the biochemical changes induced by cerebral I/R in irradiated rat brain and serum (Abd-El-Fattah et al., 2010).

Another investigation demonstrated that CoQ10 reduces brain lactate accumulation, protects against ATP depletion and improved

cellular respiratory activity in the endothelin rat model of cerebral ischemia. Thus, its ability to preserve mitochondrial function and facilitate neuronal ATP synthesis can lead to the reduction of anaerobic lactate production and brain LDH activity. It may also regulate cytosolic Ca^{2+} homeostasis via modulation of energy generation (Ostrowski, 2000; Almaas et al., 2002).

CoQ10 has also been found to prevent lipid peroxidation, a major cause of damage by free oxygen radicals. In addition, transient cerebral ischemia leads to decrease in tissue levels of CoQ10 (Mellors and Tappel 1966).

Result of another study shows that pretreatment with CoQ10 diminishes neuronal damage in the cerebral and hippocampal cortex in the endothelin-1 (ET-1) model of ischemia (Ostrowski et al., 1998). In addition, according to a recent study, coenzyme Q10 therapy involves resistance against oxidative stress which can improve the brain bioenergetics, when supplemented during reperfusion after ischemic brain injury (Horecky et al., 2011). In an experimental model of diabetes and ischemia, it was concluded that pretreatment with CoQ10, 10 mg/kg IP for 7 days reduce activity of a key enzyme (CPP32) involved in apoptotic cell death (Piotrowski et al., 2000). Another study also shows the positive effect of Coenzyme Q10 on ischemia and neuronal damage in an experimental traumatic brain-injury model in rats. Accordingly, administration of CoQ10 after trauma was shown to be protective because it significantly lowers the increased malondialdehyde (MDA) levels. Histopathological results of the mentioned study also showed a statistically significant difference between the CoQ10 and the other trauma-subjected groups (Kalayci et al., 2011).

To date, there are a few reports of histomorphologic investigations on the effect of CoQ10 in ischemia/reperfusion model of rat. According to our data, CoQ10 administration (100 mg/kg) led to a significant decrease in neuronal loss caused by ischemia/reperfusion. The mentioned dose of CoQ10 administration showed also a positive effect on diameter of cells in comparison with that in ischemia group. It seems that the neurotrophic effect of CoQ10 is able to reduce the severity of lesions in the hippocampus following transient global ischemia/reperfusion. Further studies might be required in order to investigate the neuroprotective and neurogenesis effects of this supplement on hippocampus before evaluation of these results in clinical settings.

Conclusions

Our results indicate the neurotrophic effect of CoQ10 on hippocampal neurons against damages caused by I/R. Our findings are in agreement with several previous reports. In conclusion, treatment with CoQ10 can be tested as a pharmaceutical approach to reduce the destructive effects of ischemia/reperfusion on hippocampus.

Acknowledgements

We would like to offer our special thanks to the research council of Tehran University of Medical Science, Islamic Azad University-Pharmaceutical Sciences Branch, and Azad University of Medical Sciences Research Centre for their support of this study.

References

Abd-El-Fattah AA, El-Sawalhi MM, Rashed ER, El-Ghazaly MA. Possible role of vitamin E, coenzyme Q10 and rutin in protection against cerebral ischemia/reperfusion injury in irradiated rats. Int. J. Radiat. Biol 2010; 86:1070-78.

Aliev G, Smith MA, de la Torre JC, Perry G. Mitochondria as a primary target for vascular hypoperfusion and oxidative stress in Alzheimer's disease. Mitochondrion 2004; 4:649-63.

Aliev G, Palacios HH, Walrafen B, Lipsitt AE, Obrenovich ME, Morales L. Brain mitochondria as a primary target in the development of treatment strategies for Alzheimer disease. Int. J. Biochem. Cell. Biol 2009; 41:1989-2004.

Aliev G, Obrenovich ME, Reddy VP, et al. Antioxidant therapy in Alzheimer's disease: theory and practice. Mini Rev. Med. Chem 2008; 8:1395.

Aliev G, Liu J, Shenk JC, et al. Neuronal mitochondrial amelioration by feeding acetyl-L-carnitine and lipoic acid to aged rats. J. Cell. Mol. Med 2009; 13:320-33.

Almaas R, Saugstad OD, Pleasure D, Rootwelt T. Neuronal formation of free radicals plays a minor role in hypoxic cell death in human NT2-N neurons. Pediatr. Res 2002; 51:136-43.

Brongholi K, Souza DG, Bainy AC, Dafre AL, Tasca CI. Oxygen–glucose deprivation decreases glutathione levels and glutamate uptake in rat hippocampal slices. Brain Res 2006; 1083:211-18.

Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. J. Cereb. Blood Flow Metab 2001; 21:2-14.

Dhanasekaran M, Ren J. The emerging role of coenzyme Q-10 in aging, neurodegeneration, cardiovascular disease, cancer and diabetes mellitus. Curr. Neurovasc. Res 2005; 2:447-59.

E Obrenovich M, Li Y, Parvathaneni K, et al. Antioxidants in health, disease and aging. CNS Neurol Disord Drug Targets 2011; 10:192-207.

Folkers K, Langsjoen P, Willis R, et al. Lovastatin decreases coenzyme Q levels in humans. Proc. Natl. Acad. Sci. U.S.A 1990; 87:8931-34.

Hammerman C, Goldschmidt D, Caplan M, et al. Protective effect of bilirubin in ischemia-reperfusion injury in the rat intestine. J. Pediatr. Gastroenterol. Nutr 2002; 35:344-49.

Harukuni I, Bhardwaj A. Mechanisms of brain injury after global cerebral ischemia. Neurol. Clin 2006; 24:1-21.

Horecky J, Gvozdjakova A, Kucharska J, et al. Effects of Coenzyme Q and Creatine Supplementation on Brain Energy Metabolism in Rats Exposed to Chronic Cerebral Hypoperfusion. Curr. Alzheimer Res 2011; 8:868-75.

Kalayci M, Unal MM, Gul S, et al. Effect of Coenzyme Q10 on ischemia and neuronal damage in an experimental traumatic brain-injury model in rats. BMC Neurosci 2011; 12:75.

Khalil AA, Aziz FA, Hall JC. Reperfusion injury. Plast. Reconstr. Surg 2006; 117:1024-33.

Lipton P, Lobner D. Mechanisms of intracellular calcium accumulation in the CA1 region of rat hippocampus during anoxia in vitro. Stroke 1990; 21:III60-4.

Mancuso M, Orsucci D, Volpi L, Calsolaro V, Siciliano G. Coenzyme Q10 in neuromuscular and neurodegenerative disorders. Curr. Drug Targets 2010; 11:111-21.

Mellors Aa, Tappel A. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. J. Biol. Chem 1966; 241:4353-56.

Mori T, Asano T, Matsui T, et al. Intraluminal increase of superoxide anion following transient focal cerebral ischemia in rats. Brain Res 1999; 816:350-57.

Nakamura T, Minamisawa H, Katayama Y, et al. Increased intracellular Ca< sup> 2+</sup> concentration in the hippocampal CA1 area during global ischemia and reperfusion in the rat: a possible cause of delayed neuronal death. Neuroscience 1999; 88:57-67.

Obrenovich ME, Nair NG, Beyaz A, Aliev G, Reddy VP. The role of polyphenolic antioxidants in health, disease, and aging. Rejuvenation Res 2010; 13:631-43.

Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. Annu. Rev. Pharmacol. Toxicol 2007; 47:143-83.

Ostrowski R, Piotrowski P, Pańkowska T, Smiałek M. Evaluation of morphological changes after treatment with coenzyme Q10 (CoQ10) in endothelin-1 induced experimental ischemia in the rat. Folia. Neuropathol 1998;36:185.

Ostrowski RP. Effect of coenzyme Q< sub> 10</sub> on biochemical and morphological changes in experimental ischemia in the rat brain. Brain Res. Bull 2000;53:399-407.

Piotrowski P, Wierzbicka K, Smiałek M. Neuronal death in the rat hippocampus in experimental diabetes and cerebral ischaemia treated with antioxidants. Folia Neuropathol 2000;39:147-54.

Pourahmad J, Hosseini MJ. Application of isolated mitochondria in toxicological and clinical studies. Iran J

Pharm Res 2012;11:703-04.

Quinzii CM, DiMauro S, Hirano M. Human coenzyme Q10 deficiency. Neurochem. Res 2007; 32:723-27.

Robertson LT. Memory and the brain. J Dent Educ 2002; 66:30-42.

Rothman SM, Olney JW. Glutamate and the pathophysiology of hypoxic–ischemic brain damage. Ann. Neurol 1986; 19:105-11.

Sari S, Hashemi M, Mahdian R, Parivar K, Rezayat M. The Effect of Pentoxifylline on bcl-2 Gene Expression Changes in Hippocampus after Ischemia-Reperfusion in Wistar Rats by a Quatitative RT-PCR Method. Iran J Pharm Res. 2013;12(3):495-501.

Schurr A, Rigor BM. The mechanism of cerebral hypoxic-ischemic damage. Hippocampus 1992; 2:221-28.

Siesjö BK, Katsura K, Kristian T, Li P-A, Siesjö P. Molecular mechanisms of acidosis-mediated damage, Mechanisms of Secondary Brain Damage in Cerebral Ischemia and Trauma. Springer; 1996; 8-14.

Simone CB, Simone NL, Simone V. Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, part 1. Altern Ther Health Med 2007; 13:22.

Smith CUM. Elements of Molecular Neurobiology. 3rd ed. John Wiley and sons, Ltd., 2003: 477-506.

Stys PK, Waxman SG, Ransom BR. Na^{+}-Ca^{2+} exchanger mediates Ca^{2+} influx during anoxia in mammalian central nervous system white matter. Annals of neurology 1991; 30:375-80.

Vančová Og, Bačiak L, Kašparová S, et al. In vivo and in vitro assessment of brain bioenergetics in aging rats. J. Cell. Mol. Med 2010; 14:2667-74.

Wu B, Ootani A, Iwakiri R, et al. Ischemic preconditioning attenuates ischemia-reperfusion-induced mucosal apoptosis by inhibiting the mitochondria-dependent pathway in rat small intestine. Am. J. Physiol. Gastrointest. Liver Physiol 2004; 286:G580-G87.

Yousuf S, Atif F, Hoda N, et al. Oral supplementation of Majun Baladar ameliorates antioxidant enzyme activities in cerebral ischaemic damage. Basic Clin. Pharmacol. Toxicol 2007; 101:246-53.