Vol 5 | Issue 2 | 2014 | 100-104.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

EFFCTS OF TRADITIONAL CHINESE MEDICINE IN CEREBRAL ISCHEMIA

***Abhishek Kumar and Amanpreet Kaur**

Rayat & Bahra institute of pharmacy, Sahauran, kharar, Distt-Mohali (Punjab), India.

ABSTRACT

Cerebral Ischemia is the major cause of high morbidity and mortality for both developed and developing countries. It is a serious neurological disease in which sudden loss of brain function resulting from interference with the blood supply to the central nervous system. Because of its very few clinical, social, and economic implications it demands a significant effort from both basic scientists and clinicians to adopt suitable preventive measures and successful therapies. Till date only the recombinant tissue plasminogen activator (rtPA) are FDA-approved drug is available for the treatment of ischemic stroke but due to short therapeutic window, use of them is limited. Cerebral ischemia is a complex pathological process involving a series of mechanisms, and a framework for the development of neuroprotectants from traditional herb medicine is a promising treatment for cerebral ischemia. Chinese medicinal herbs exert neuroprotective effects on cerebral ischemia. Natural compounds with the effects of anti-oxidation, anti-inflammation, exhibit preventive or therapeutic effects on experimental ischemic brain injury. According to the pharmacological mechanisms underlying neuroprotection, we evaluated natural products from traditional medicinal herbs that exhibit protective effects on ischemic brain injury and characterized the promising targets.

Keywords: Ischemia, rt-PA, FDA, Neuroprotection.

INTRODUCTION

Cerebral ischemia takes place when the amount of oxygen and other nutrients supplied by blood flow is insufficient to meet the metabolic demands of brain tissue [1]. In cerebral ischemia there is an ischemic gradient that can be divided into the penumbra, which is located in more peripheral zones and the core, which is the central ischemic zone. In the penumbra, functional alterations occur in the neurons and glial cells. Due to their dependence on the oxidative metabolism of glucose for energy neurons are most vulnerable to hypoxia [2].

Cerebral ischemia leads to a cascade of various pathophysiological events, which contribute to ischemic cell damage. Among the various pathophysiological changes which are postulated to occur as a response to stroke are free radical production, disruption of sodium and calcium influx, excitotoxicity, enzymatic changes, stimulation of the inflammatory process, endothelin (ET) release, activation of platelets and leukocytes, endothelial dysfunction and delayed coagulation. All of these pathophysiological processes leads to the brain injury following the onset of stroke [3].

Herbal drugs have been gained a lot of acceptance in recent years and become potential candidates for prophylactic treatment because of fewer side effects, high therapeutic window and are economical. A large number of traditional Chinese medicines have been used in both animal model of stroke and human patients and found to be effective. However there is comprehensive review available related to use of herbal medicine in prophylaxis and prevention and treatment of stroke [4-6].

Anti inflammatory effect of Chinese medicine

Activation of various inflammatory processes accounts for the generation of ischemic stroke [7], after the onset of cerebral ischemia, necrotic neuron death and energy depletion in the local ischemic area starts the inflammatory processes. The reperfusion generates reactive oxygen species (ROS) which induce the production of chemokines and cytokines leading peripheral leukocytes to influx into the cerebral parenchyma and activate endogenous microglia. Then in the inflammatory process, cellular immunity, adhesion molecules, inflammatory mediators, transcriptional factors participates. Anti-inflammatory drugs that prevents specific steps of the inflammatory cascade is a new planning for improving results after ischemic stroke [8,9]. The anti-inflammatory agents, including various types of

Corresponding Author: **Abhishek Kumar Email:-** abhishekkaundal89@gmail.com

natural products used in Chinese medicine, have been shown to be able to treat or prevent ischemic stroke, by decreasing the neurological deficiency and infract area [10]. These types of natural products are documented as anti-inflammatory, anti-apoptotic, anti-oxidative and neuro-functional regulatory agents. Few active compounds isolated from these herbs have been identified and demonstrated to have neuroprotective actions. Some example of these compounds are andrographolide isolated from *Andrographis paniculata*, oxymatrine isolated from Sophora flavescens, quercetin isolated from *Sophora japonica*, ferulic acid isolated from both *Angelica sinensis* and *Ligusticum wallichii*, tetramethylpyrazine isolated from *Ligusticum wallichii*, paeonol and paeoniflorin isolated from *Paeonia lactiflora*, shikonin isolated from *Lithospermum erythrorhizon*, vanillin, 4-hydroxybenzyl alcohol and 4-hydroxybenzyl aldehyde isolated from Gastrodia elata, puerarin from Radix Puerariae, polydatin and emodin-8-O-b-D-glucoside isolated from *Polygonum cuspidatum*, tanshinone IIA isolated from *Salviae miltiorrhiza*, wogonin isolated from *Scutellaria baicalensis* and apocynin isolated from *Picrorhiza kurroa* [11].

Anti-oxidative effect of Chinese medicine

The main cause of aging, coronary heart disease, stroke, diabetes mellitus, rheumatism and cancer are known to be Free radicals [12-14]. It has been reported that in cellular injuries, reactive oxygen species (ROS) such as O_2 − (superoxide anion), OH (hydroxyl radical), H_2O_2 (hydrogen peroxide) and $1O_2$ (singlet oxygen), play an important role and also initiate the peroxidation of polyunsaturated fatty acids in biological membranes [13-15]. The ROS causes DNA damage, protein damage and oxidation of enzymes in the human beings [13-17]. By a natural antioxidant defence system involving enzymatic and non-enzymatic mechanisms, aerobic organisms are protected from oxygen toxicity [18,19]. It has been reported that various types of non-enzymatic antioxidants such as ascorbic acid, tocopherol, carotenoids, flavonoids and micronutrients such as zinc and selenium are present in the high ammount in a number of medicinal plants [20,21]. Different type of Natural antioxidants derived from plant products, such as herbs, legumes, tea and *Panax ginseng* were reported to prevention and treatment of oxidative stresses [22-24]. Traditionally, *A. sinensis* AS (Umbelliferae) is mainly used for the treatment of anaemia, gynaecological disorders, hypertension, headache, asthma and rheumatism. The fruit part of *L. barbarum* LB (Solanaceae) is mainly used as antipyretic, antiinflammation, pneumonia, nephritis, diuretic and antisenile agents. Hot water extracts of various medicinal plants are commonly used to prepare various traditional Chinese medicated diets [25].

It has been reported that during ischemia/reperfusion, a burst of ROS is produced which leads to the oxidation of lipids, proteins and DNA and subsequently causes cellular damage and apoptosis [26,27]. Therefore, much attention has been paid to the prevention of brain injury after ischemia/ reperfusion via inhibiting bursts of ROS. Many natural compounds with antioxidant ability, such as flavonoids from *Scutellaria baicalensis* Georgi [28,29]. Curcuma Oil (isolated from powdered rhizomes of *Curcumalonga Linn*) [30]. *Ginkgo biloba* extract EGb761 [31] and Cinnamophilin (isolated from *Cinnamomum philippinense*) [32] exhibit significant neuroprotective effects when they are administered before cerebral ischemia occurs, but the related mechanisms or targets have been identified for only a few compounds. For instance, flavonoids from *Scutellaria baicalensis* Georgi, when either pretreated or post-treated, are demonstrated to decrease levels of malondialdehyde (MDA) and increase the level of superoxide dismutase (SOD) in the ischemic brains of mice. Aside from the anti-oxidant effects, flavonoids are also found to inhibit platelet aggregation, which is important to improve ischemic brain injury [28]. Pretreatment with curcuma oil, isolated from powdered rhizomes of *Curcuma longa* Linn, significantly reduces the levels of NO, ROS, ONOO–, and mitochondrial membrane potential [30].

There are more and more reports focusing on the precise mechanisms or direct targets of natural antioxidant compounds protecting against cerebral ischemia. Some natural compounds exhibit direct regulatory effects on endogenous antioxidant enzyme systems. For example, Heme oxygenase (HO) is the rate-limiting enzyme for catabolism of the prooxidant heme. Two isoforms of HO exist: an inducible form (HO-1) and a constitutively expressed form (HO-2). HO-1 can be induced in response to various noxious stimuli (such as hypoxia and oxidative stress) and is considered a gene that protects against I/R injury [34,35]. CA, a catechol-type electrophilic compound found in the herb rosemary obtained from *Rosmarinus officinalis*, is shown to be neuroprotective when injected 1 h prior to MCAO in mice. As a representative electrophile, CA can induce the expression of a set of antioxidant enzymes, including heme oxygenase-1 (HO-1), NADPH quinine oxidoreductase 1 (NQO1), and c-glutamyl cysteine ligase (c-GCL). CAs become electrophilic quinones upon oxidation, with protective effects against neuronal oxidative stress and excitotoxicity via binding to specific Keap1 cysteine residues as a direct drug target, and then activating the Keap1/Nrf2 transcriptional pathway. The most attractive advantage of this agent is that it is a pro-electrophilic compound that can be activated by the microenvironment of oxidative stress and only becomes electrophilic at or near the site of ischemic brain tissues, with lower toxicity to normal tissues [29]. The protective effect of *Ginkgo biloba* extract on cerebral ischemia can be abolished in HO-1 knockout mice, suggesting that HO-1 is the key target of neuroprotection against free radical damage. In cerebral ischemia, nitro oxide (NO) plays both a protective and a destructive role at different stages of this complex process. The beneficial effects of NO are related to the small amount of NO produced by endothelial nitric oxide synthase (eNOS), which produces significant effects on the maintenance of cerebral blood flow, prevention of neuronal injury by activation of the GC-cGMP-PKG pathway, and inhibition of platelet as well as leukocyte adhesion, and therefore protects against cerebral ischemia [36,37]. Refined Qing Kai Ling (RQKL), an improved

injectable multi-component preparation derived from Qing Kai Ling, shows neuroprotection in MCAO rats by relieving vascular endothelial cell damage as well as inhibiting inflammation. More importantly, RQKL was able to stimulate the post-ischemic expression of eNOS, which might be an essential part of the neuroprotective mechanisms of RQKL [38]. However, the large amount of NO, which is derived from inducible nitric oxide synthase (iNOS), harms neurons by producing peroxynitrite after the reaction with superoxide. Peroxynitrite can inhibit the mitochondrial respiratory chain, which implicates the involvement of ATP loss and eventually leads to irreversible cellular damage [38,40]. Tetrahydroxystilbene glucoside (TSG), an active component of the rhizome extract from *Polygomum multiflorum*, has been reported to attenuate intracellular ROS generation and mitochondrial membrane potential dissipation caused by ischemia/ reperfusion. Interestingly, it can directly upregulate the expression of sirt1, which is a class III histone deacetyltransferase that promotes cell survival and subsequently reduces the expression and activity of iNOS. This in turn induces the increase in NO production as well as peroxynitrite formation and results in apoptotic cell death by inhibiting the phosphorylation and subsequent degradation of I-κB, thereby hampering the DNA binding of nuclear factor kappa-B (NF-κB) by sirt1 activation.[41] More evidence has emphasized the significance of sirt1 in promoting cell survival, regulating lifespan and inhibiting inflammation. Recently, sirt1 has been introduced for the therapy of neurodegenerative diseases [42,43].

Many natural compounds such as resveratrol, butein and quercetin, which are known as anti-aging agents, have been found to directly activate sirt1 [44] suggesting that sirt1 may be the direct target of many herbal components that exhibit anti-aging effects. Phosphoinositide 3-kinases (PI3K)/Akt regulate the survival response against oxidative stress-associated neuronal apoptosis, which is determined by the balance between the activity of PI3K and the phosphatase and tensin homolog (PTEN). Activation of Akt promotes cell survival and suppresses apoptosis by inhibition of several downstream substrates, including glycogen synthase kinase-3β (GSK3β). PTEN is a major negative regulator of the PI3K/Akt signaling pathway and has been demonstrated to act as an important mediator of ROS production and mitochondria-dependent

Apoptosis [45], a recent study reveals that Baicalein (Bai), on flavonoid extracted from *Scutellaria baicalensis* Georgi, when administered either prior to or after ischemia, can significantly protect against brain injury. Similarly, incubation with Bai reverses the rapid PTEN dephosphorylation after oxygenglucose deprivation (OGD) in cultured hippocampal neurons [46].

Anti- apoptotic property of Chinese medicine

Ischemic cerebral injury is known to induce histopathological damage and related neurological deficits, leading to the activation of complex neurochemical cascades of cell death, which are primarily expressed as apoptosis. In principle, these apoptotic cascades are reversible and form an important aspect of the penumbra concept, a major target of therapeutic interventions.

In general, ischemia and reperfusion induced neuronal apoptosis can be classified into two types: caspase-dependent and caspase-independent pathways. Caspases are intracellular proteases that function as initiators and effectors of apoptosis. When activated, caspases cleave a variety of intracellular proteins, including major structural elements in the cytoplasm and nucleus, components of the DNA repair machinery, and a number of protein kinases [47]. Thus, caspases may be effective against ischemia-induced neuronal cell apoptosis by blocking apoptotic cascades with appropriate drugs. An alkaloid-free ethyl acetate, extracted from the root of *Sophora flavescens*, has been reported to protect against focal cerebral ischemia by decreasing DNA fragmentation and inhibiting caspase-3 activity directly [48]. In that study, it was found that caspase-independent programmed cell death, mediated by the translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus, plays a key role in ischemia. During ischemia and reperfusion injury, in the case of massive and irreparable DNA damage, overactivation of Poly (ADP-ribose) Polymerase-1 (PARP-1) can lead to necrotic cell death caused by the depletion of NAD+ and ATP, as well as enhance AIF release from mitochondria [49]. A series of PARP-1 antagonists have been developed, some of which have become promising drug candidates [50,51]. However, the characterization of natural compounds regulating PARP-1 remains to be elucidated. It is thought that berberine performs its anti-apoptotic effect by inhibiting both caspase-dependent and independent pathways [52]. Synergistic inhibition of caspase-dependent and independent neuronal cell apoptosis is more likely to be effective as a therapeutic approach for the treatment of ischemia. Numerous herb drugs have been demonstrated to act on anti-apoptotic pathways, such as Bcl-2 family proteins. The abundant expression of Bcl-xL protein in the adult brain is known to suppress activation of procaspase 9 by forming a complex with Apaf1 and to prevent the release of cytochrome c from mitochondria, thus maintaining cell viability. Therefore, Bcl-xL becomes a promising target for drug intervention to reduce cell apoptosis [53]. Pretreatment with 4-hydroxybenzyl alcohol 30 min before ischemia, one of the major active phenolic constituents of *Gastrodia elata Blume*, could antagonize cerebral ischemia by increasing Bcl-2 expression and inhibiting caspase-3 activity, leading to the amelioration of cell apoptosis in ischemic regions. Direct evidence from preclinical research shows that Ginsenoside Rb1 (gRb1) regulates the anti-apoptosis signaling pathway, which stimulates the expression of mitochondrion-associated anti-apoptotic factor

Bcl-xL through the use of reporter plasmids. The transcription factor signal transducer and activator of transcription 5 (Stat5) are known to activate Bcl-xL family proteins via binding to the bcl-xL promoter. The Stat5 responsive element in the bcl-xL promoter becomes active in response to gRb1 treatment, suggesting that the Stat5

pathway participates in anti-apoptotic effects by regulating Bcl-2 indirectly through activation of gRb1. Molecular pharmacology research in this direction should elucidate the direct target of natural drugs that regulate apoptotic signaling pathways [10]

DISCUSSION

Traditional Chinese medicine (TCM) has been extensively studied in stroke therapy. There are more than 100 traditional Chinese medicines which have been studied for stroke treatment both in animals as well as in patients. A number of commercial stroke treatments based on TCM have been introduced into the market recently after extensive pharmacological research and clinical trials. Similar to western medicine, chinese medicine therapies are based on the pathophysiology of stroke and are classified as antioxidants, anti-inflammatory, anti-apoptotic etc. In conclusion, development of protective agents from traditional herb medicine is a promising direction in the treatment of ischemic cerebral injury and related neurodegenerative diseases. In the future, more attention should be paid to natural compounds that can transverse the BBB and have wide therapeutic time windows, clear pharmacological targets and fewer side effects.

REFERENCES

1. Graham SH, Hickey RW. Molecular pathophysiology of stroke. *Neuropsychopharmacology, The Fifth Generation of Progress.* 2002, 1310-1327.
2. Gilgun-Sherk Y, Zivrosenbaum, Eldadmelamed, Offen D. Antioxidant Therapy in Acute Central Nervous System Injury, Current State. *The American Society for Pharmacology and Experimental Therapeutics*, 54(2), 2002, 271-278.
3. Gupta YK, Briyal S. Animal models of cerebral ischemia for evaluation of drugs. *Indian J Physiol Pharmacol*, 48(4), 2004, 379–394.
4. Fisher M, Schaebitz W. An overview of acute stroke therapy, past, present, and future. *Arch Intern Med*, 160, 2000, 3196–3206.
5. Jonas S. Prophylactic pharmacologic neuroprotection against focal cerebral ischemia. *Ann N Y Acad Sci*, 765, 21(5), 1995, 6–7.
6. Chaudhary G, Sharma U, Jagannathan NR, Gupta YK. Evaluation of Withania somnifera in a middle cerebral artery occlusion model of stroke in rats. *Clin Exp Pharmacol Physiol*, 30, 2003, 399–404.
7. Ahmad M, Graham S.H. Inflammation after stroke, mechanisms and therapeutic approaches. *Transl Stroke Res*, 1(2), 2010, 74-84.
8. Stoll G, Kleinschnitz C, Nieswandt B. Combating innate inflammation, a new paradigm for acute treatment of stroke? *Ann N Y Acad Sci*, 1207, 2010, 149-154.
9. del Zoppo GJ. Acute anti-inflammatory approaches to ischemic stroke. *Ann N Y Acad Sci*, 1207, 2010, 143-148.
10. Wu PF, Zhang Z, Wang F, Chen JG. Natural compounds from traditional medicinal herbs in the treatment of cerebral ischemia/reperfusion injury. *Acta Pharmacol Sin*, 31(12), 2010, 1523-1531.
11. Su S, Hsieh C. Anti- inflammatory effects of Chinese medicinal herbs on cerebral ischemia. *Chinese Medicine*, 6(26), 2011, 1-9.
12. Wong SH, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW. Lipoperoxide in plasma as measured by liquid-chromatographic separation of malondialdehyde thiobarbituric acid adduct. *Clin Chem*, 33, 1987, 214– 220.
13. Halliwell B. Antioxidants and human disease, A general introduction. *Nutr Rev*, 55, 1997, 44–52.
14. Droge W. Free radicals in physiological control of cell function. *Physiol Rev*, 82, 2002, 47–95.
15. Compori M. Lipid peroxidation and cellular damage in toxic liver injury. *Lab Invest*, 53, 1985, 599–620.
16. Bartold PM, Wiebkin OW, Thonard JC. The effects of oxygen-derived free radicals on gingival proteoglycans and hyaluronic acid. *J Periodontol Res*, 19, 1984, 390–400.
17. Varani J, Fligiel SEG, Till GO, Kunkel RG, Ryan VS, Ward PA. Pulmonary endothelial cell killing by human neutrophils, possible involvement of hydroxyl radical. *Lab Invest*, 53, 1985, 656–663.
18. Cotgreave I, Moldeus P, Orrenius S. Host biochemical defense mechanisms against prooxidants. *Ann Rev Pharmacol Toxicol*, 28, 1988, 189–212.
19. Ames BN, Shigenaga MS, Hagen T. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA*, 90, 1993, 7915–7922.
20. Polidori M.C, Stahl W, Eichler O, Niestroj I, Sies H. Profiles of antioxidants in human plasma. *Free Radic Biol Med*, 30, 2001, 456–462.
21. Lin CC, Wu SJ, Wang JS, Yang JJ, Chang CH. Evaluation of the antioxidant activity of legumes. *Pharm Biol*, 39, 2001, 300–304.
22. Xuejiang W, Ichikawa H, Konishi T. Antioxidant potential of Qizhu Tang, a Chinese herbal medicine, and the effect on cerebral oxidative damage after ischemia reperfusion in rats. *Biol Pharm Bull*, 24, 2001, 558–563.
23. Roedig-Penman A, Gordon M.H. Antioxidant properties of catechins and green tea extracts in model food emulsions. *J Agric Food Chem*, 45, 1997, 4267–4270.
24. Keum YS, Park KK, Lee JM, Chun YS, Suh YJ. Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Lett*, 150, 2000, 41– 48.
25. WU SJ, Ng LT, Lin CC. Antioxidant Activities of Some Common Ingredients of Traditional Chinese Medicine, *Angelica sinensis*, *Lycium barbarum* and *Poria cocos. Phytotherapy Research*, 18, 2004, 1008–1012.

26. Niizuma K, Endo H, Chan PH. Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival. *J Neurochem*, 109, 2009, 133–8.
27. Crack PJ, Taylor JM. Reactive oxygen species and the modulation of stroke. *Free Radic Biol Med*, 38, 2005, 1433–44.
28. Zhang Y, Wang X, Wang X, Xu Z, Liu Z, Ni Q. (2006). Protective effect of flavonoids from *Scutellaria baicalensis* Georgi on cerebral ischemia injury. *J Ethnopharmacol*, 108, 355–60.
29. Satoh T, Kosaka K, Itoh K, Kobayashi A, Yamamoto M, Shimojo Y. Carnosic acid, a catechol-type electrophilic compound, protects neurons both *in vitro* and *in vivo* through activation of the Keap1/ Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. *J Neurochem*, 104, 2008, 1116–31.
30. Rathore P, Dohare P, Varma S, Ray A, Sharma U, Jagannathan N.R. (). Curcuma oil, reduces early accumulation of oxidative product and is anti-apoptogenic in transient focal ischemia in rat brain. Neurochem Res, 33, 2008, 1672–82.
31. Saleem S, Zhuang H, Biswal S, Christen Y, Doré S. *Ginkgo biloba* extract neuroprotective action is dependent on heme oxygenase 1 in ischemic reperfusion brain injury. *Stroke*, 39, 2008, 3389–96.
32. Ee EJ, Chen HY, Lee MY, Chen TY, Hsu YS, Hu YL. Cinnamophilin reduces oxidative damage and protects against transient focal cerebral ischemia in mice. *Free Radic Biol Med*, 39, 2005, 495–510.
33. Imura T, Weinstein PR, Massa S.M, Panter S, Sharp FR. Heme oxygenase-1 (HO-1) protein induction in rat brain following focal ischemia. *Brain Res Mol Brain Res*, 37, 1996, 201–8.
34. Doré S. (). Decreased activity of the antioxidant heme oxygenase enzyme, implications in ischemia and in Alzheimer's disease. *Free Radic Biol Med*, 32, 2002, 1276–82.
35. Li RC, Saleem S, Zhen G, Cao W, Zhuang H, Lee J. Hemehemopexin complex attenuates neuronal cell death and stroke damage. *J Cereb Blood Flow Metab*, 29, 2009, 953–64.
36. Endres M, Laufs U, Liao J.K, Moskowitz M.A. Targeting eNOS for stroke protection. *Trends Neurosci*, 27, 283–9.
37. Ha KS, Kim KM, Kwon YG, Bai SK, Nam WD, Yoo YM. Nitric oxide prevents 6-hydroxydopamine-induced apoptosis in PC12 cells through cGMP-dependent PI3 kinase/Akt activation. *FASEB J*, 17, 2003, 1036–47.
38. Hua Q, Zhu X, Li P, Tang H, Cai D, Xu Y. Refined Qing Kai Ling, traditional Chinese medicinal preparation, reduces ischemic stroke-induced infarct size and neurological deficits and increases expression of endothelial nitric oxide synthase. *Biol Pharm Bull*, 31, 2008, 633–7.
39. Bolaños JP. Almeida A. Roles of nitric oxide in brain hypoxia-ischemia. *Biochim Biophys Acta*, 1411, 1999, 415–36.
40. Del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. Inflammation and stroke, putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol*, 10, 2000, 95–112.
41. Wang T, Gu J, Wu PF, Wang F, Xiong Z, Yang YJ. Protection by tetrahydroxystilbene glucoside against cerebral ischemia, involvement of JNK, SIRT1, and NF-kappaB pathways and inhibition of intracellular ROS/RNS generation. *Free Radic Biol Med*, 47, 2009, 229–40.
42. Lavu S, Boss O, Elliott PJ, Lambert PD. Sirtuins — novel therapeutic targets to treat age-associated diseases. *Nat Rev Drug Discov*, 7, 2008, 841–53.
43. Tang BL, Chua CE. SIRT1 and neuronal diseases. *Mol Aspects Med*, 29, 2008, 187–200.
44. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature*, 425, 2003, 191–6.
45. Waite KA, Eng C. Protean PTEN, form and function. *Am J Hum Genet*, 70, 2002, 829–44.
46. Wu PF, Zhang Z, Wang F, Chen J. Natural compounds from traditional medicinal herbs in the treatment of cerebral ischemia/reperfusion injury. Acta Pharmacologica Sinica 31, 2010, 1523–1531.
47. Van Wijk SJ, Hageman GJ. Poly (ADP-ribose) polymerase-1 mediated caspase-independent cell death after ischemia/reperfusion. *Free Radic Biol Med*, 39, 2005, 81–90.
48. Zhang WT, Ruan JL, Wu PF, Jiang FC, Zhang LN, Fang W. Design, synthesis, and cytoprotective effect of 2-aminothiazole analogues as potent poly(adp-ribose) polymerase-1 inhibitors. *J Med Chem*, 52, 2009, 718–25.
49. Ferraris D, Ficco R.P, Dain D, Ginski M, Lautar S, Lee-Wisdom K. Design and synthesis of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. Part 4, Biological evaluation of imidazobenzodiazepines as potent PARP-1 inhibitors for treatment of ischemic injuries. *Bioorg Med Chem* , 11, 2003, 3695–707.
50. Ye M, Fu S, Pi R, He F. Neuropharmacological and pharmacokinetic properties of berberine, a review of recent research. *J Pharm Pharmacol*, 61, 2009, 831–7.
51. Rami A, Bechmann I, Stehle JH. Exploiting endogenous anti-apoptotic proteins for novel therapeutic strategies in cerebral ischemia. *Prog Neurobiol*, 85, 2008, 273–96.
52. Yu SS, Zhao J, Zheng WP, Zhao Y. Neuroprotective effect of 4-hydroxybenzyl alcohol against transient focal cerebral ischemia via antiapoptosis in rats. Brain Res, 1308, 2010, 167–75.
53. Zhang B, Hata R, Zhu P, Sato K, Wen TC, Yang L. Prevention of ischemic neuronal death by intravenous infusion of a ginseng saponin, ginsenoside Rb(1), that upregulates Bcl-xL expression. *J Cereb Blood Flow Metab*, 26, 2006, 708–21.