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Full Length Research Paper

The antioxidant effect of picroside Ⅱ and the optimizing of therapeutic dose and time window in cerebral ischemic injury in rats

Meizeng Zhang[1]*, Pang Fangfang[1], Rui Zhang[2], Li Zhao[1] and Yiling Wu[3]

[1]Department of Neurology, Affiliated Hospital of Qingdao University Medical College, Qingdao 266003, China
[2]Department of ICU, Affiliated Hospital of Qingdao University Medical College, Qingdao 266003, China
[3]Department of Rehabilitation, Affiliated Hospital of Qingdao University Medical College, Qingdao 266003, China

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To optimize the antioxidant effect and the best therapeutic dose and time window of picroside Ⅱ in cerebral ischemic injury in rats by orthogonal test. The forebrain ischemia models were established by bilateral common carotid artery occlusion (BCCAO) methods. The successful rat models were randomly divided into 16 groups according to orthogonal experimental design of [$L_{16}(4^5)$] and treated by injecting picroside Ⅱ intraperitoneally with different doses at various times. The concentrations of malondialdehyde (MDA), nitric oxide (NO) and hydrogen per oxide (H_2O_2) in serum and brain tissue were respectively determined by thiobarbituric acid assay, nitratase reductase assay and chemiluminescence's immunoassay. The optimized compositions of the therapeutic dose and time window of picroside Ⅱ in cerebral ischemic injury were (1) ischemia 1.5h with 10mg/kg, 1.5h with 20mg/kg and 1.5h with 10mg/kg body weight according to the expressions of MDA, NO and H_2O_2 in serum respectively, (2) ischemia 1.5h with 10mg/kg, 1.5h with 20mg/kg and 1.5h with 20mg/kg body weight according to the expressions of MDA, NO and H_2O_2 in brain tissue respectively. Conclusion On the basis of the principle of the minimization of medication dose and maximization of therapeutic time window, the optimized composition of the therapeutic dose and time window in cerebral ischemic injury was injecting picroside Ⅱ intraperitoneally with 10-20mg/kg body weight at ischemia 1.5h.

Keywords: picroside Ⅱ, cerebral ischemia, therapeutic dose, time window, MDA, NO, H_2O_2, rats

INTRODUCTION

After cerebral ischemia, the body is in a state of oxidative stress due to the imbalance between oxygen radicals production and clearance in vivo (Shibata and Kobayashi, 2008; Li et al., 2010a). Oxygen radicals attack poly-unsaturated fatty acid in the biomembranes and induce lipid peroxidation to destroy the integrity of the membrane structure, inducing the disorders of the intracellular environment and apoptosis (Perluigi et al., 2010; Asano et al., 2012). Free radicals injury plays a very important role in cerebral ischemia reperfusion injury (Wang and Shuaib, 2007; Xi et al., 2007). Malondialdehyde (MDA) can reflect the equilibrium state of oxidative stress (Yuan et al., 2011) and the content of free radicals and the degree of lipid peroxidation in tissue (Tomatsuri et al., 2004; Lv et al., 2011). The aldehyde groups of MDA can cause cross-linked polymeric reaction with the amino component of phosphatidylcholine and phosphatidyl-

*Corresponding Author's E-mail: zmzcmd@163.com
Tel: +8618661808039

Table 1. Orthogonal experimental design of [$L_{16}(4^5)$]

Therapeutic dose	Ischemia 1.0h(A1)	Ischemia 1.5h(A2)	Ischemia 2.0h(A3)	Ischemia 2.5h(A4)
5mg·kg^{-1}(B1)	1.0×5	1.5×5	2.0×5	2.5×5
10mg·kg^{-1}(B2)	1.0×10	1.5×10	2.0×10	2.5×10
20mg·kg^{-1}(B3)	1.0×20	1.5×20	2.0×20	2.5×20
40mg·kg^{-1}(B4)	1.0×40	1.5×40	2.0×40	2.5×40

serine in phosphatides in nerve cell biomembrance to change the structures and functions of biomembrance (Chen et al., 2004; Amemiya et al., 2005). Lipid peroxidation in biomembrance and its products can also cause oxidative injury of membrane proteins, leading to apoptosis (Cejas et al., 2004; Liu et al., 2008). After cerebral ischemia reperfusion, the local excitatory amino acids release and activate inducible nitric oxide synthase (iNOS), resulting in an increase in nitric oxide (NO) synthesis (Choi, 1988), and NO and O^{2-} will generate a more virulent OH- and NO^{2-} to aggravate ischemia reperfusion injury (Ito et al., 2010) and induce lipid peroxidation and apoptosis or cell death (Brown, 2010; Rey et al., 2011). It has been proved that picroside Ⅱ could lessen the PC12 cell damage induced by hydrogen peroxide (H_2O_2) (Li et al., 2002; Tao et al., 2003; Guo et al., 2007) and the L-02 cell injury caused by oxidative stress (Gu et al., 2008) and raise the cell survival rate in vivo. Animal experiments showed that picroside Ⅱ could inhibit the expression of related inflammatory factors and cell apoptosis in ischemic penumbra after ischemic reperfusion injury and improve the neurobehavioral function in rats (Li et al., 2010b, 2010a,b). Our former experiments indicated that the best therapeutic effect is injecting picroside Ⅱ intraperitoneally with 20mg/kg at ischemia 1.5h after intervened by picroside Ⅱ with different doses at various times in rats (Pei et al., 2012). In this study, the authors aimed to detect the concentrations of MDA, NO and H_2O_2 in serum and brain tissue to further explore the antioxidation and the optimal therapeutic dose and time window of picroside Ⅱ in cerebral ischemic injury in rats according to the design principle of orthogonal test.

MATERIALS AND METHODS

Establishment of animal models

A total of 30 adult healthy male Wistar rats, SPF grade, weight 230-250g, were supplied by the Experiment Animal Center of Qingdao Institute for the Control of Drug Products (SCXK (LU) 20100010). All animals were acclimatized for a week in the laboratory and allowed free access to food and water in a room temperature (23±2℃) and humidity-controlled housing with natural illumination, fasting 12h before operation. Five rats were randomly selected as a sham operated group and the rest 25 rats were used to establish the forebrain ischemia models by bilateral common carotid artery occlusion (BCCAO) methods (Márquez-Martín et al., 2012). Rats in the sham operated group were experimented without BCCAO compared with those in the experimental group. The rats were anesthetized by injecting intraperitoneally 10% chloral hydrate (3ml/kg) and fixed in supine position to conduct aseptic operation. The four have not regained consciousness after operation for 2h or dead rats were excluded from the experiment, and the rest 21 successful animal models were randomly divided into model group (n=5) and treatment group (n=16).

Orthogonal experimental design

Sixteen successful BCCAO rat models were internalized into the experiment and divided randomly according to the principle of orthogonal experimental design of [$L_{16}(4^5)$] consisting of two impact factors with four impact levels (Table 1). The impact factor A is the therapeutic time window designed four levels: 1.0h, 1.5h, 2.0h, 2.5h after ischemia. The impact factor B is the therapeutic drug dose which has four levels as follows: 5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg body weight.

Intervention

Picroside Ⅱ (Tianjin Kuiqing Medical Technology Co. Ltd., CAS No: 39012-20-9, purity >98%, molecular weight: 512) was diluted into 1% solution with 0.1 mol/L PBS and injected intraperitoneally according to the corresponding designed doses in the orthogonal layout [$L_{16}(4^5)$]. After treatment for 24h, the rats were killed to detect the corresponding indexes. The sham operated group and model group rats were synchronously injected the same amount of physiological saline intraperitoneally after operation for 2h.

Specimen collection

After treatment with picroside Ⅱ for 24h, the rats were

Table 2. The results of concentrations of MDA, NO and H_2O_2 ($\bar{x} \pm s$)

Groups	n	MDA_{Serum} (mmol/L)	MDA_{Brain} (mmol/L)	NO_{Serum} (μmol/L)	NO_{Brain} (μmol/L)	H_2O_{2Serum} (mmol/L)	H_2O_{2Brain} (mmol/L)
Sham group	5	2.53±0.25	6.360±0.68	45.56±6.72	1.12±0.25	5.03±0.45	12.13±1.25
Model group	5	4.38±0.56[a]	9.60±0.82[a]	80.33±9.26[a]	2.67±0.34[a]	9.46±0.72[a]	17.36±1.56[a]
Treatment group	16	3.34±0.78[b]	7.61±1.00[b]	65.95±8.90[b]	1.74±0.65[b]	6.73±1.47[b]	15.17±1.58[b]

[a] $P<0.01$, vs the sham group; [b] $P<0.01$, vs the model group.

Table 3. [$L_{16}(4^5)$] orthogonal layout and the detection results

Test NO.	Rank NO.					Serum MDA	Brain MDA	Serum NO	Brain NO	Serum H_2O_2	Brain H_2O_2
	A	B	C	D	E						
1	1	1	1	1	1	2.27	7.62	67.50	1.88	5.46	15.06
2	1	2	2	2	2	2.58	7.44	66.67	2.13	5.38	14.80
3	1	3	3	3	3	3.11	7.30	62.22	1.93	6.21	14.61
4	1	4	4	4	4	3.16	7.54	71.11	2.05	6.30	15.08
5	2	1	2	3	4	3.34	7.19	71.67	2.11	6.72	14.29
6	2	2	1	4	3	2.61	6.81	62.11	1.05	5.11	13.61
7	2	3	4	1	2	2.32	6.06	51.39	0.65	4.65	12.14
8	2	4	3	2	1	2.73	6.22	56.94	0.96	5.46	14.23
9	3	1	3	4	2	3.86	7.16	66.67	1.08	7.65	17.31
10	3	2	4	3	1	3.13	6.39	50.56	1.04	6.17	13.70
11	3	3	1	2	4	3.09	8.20	54.17	1.28	6.18	14.04
12	3	4	2	1	3	4.05	8.70	71.33	2.78	8.05	16.38
13	4	1	4	2	3	4.93	8.49	78.22	2.34	9.83	16.71
14	4	2	3	1	4	3.82	8.56	72.62	1.80	7.66	16.04
15	4	3	2	4	1	3.88	8.30	76.25	1.93	7.76	16.56
16	4	4	1	3	2	4.51	9.70	75.83	2.77	9.02	18.17
Ⅰ	11.12	14.40	12.48	12.46	12.01	53.39	121.68	1055.2	27.78	107.61	242.7
Ⅱ	11.00	12.14	13.85	13.33	13.27						
Ⅲ	14.13	12.40	13.52	14.09	14.70						
Ⅳ	17.14	14.45	13.54	13.51	13.41						
SS	6.37	1.17	0.27	0.34	0.91						

anesthetized by injecting intraperitoneally 10% chloral hydrate (3ml/kg) and then they were opened the chest, the blood (about 4ml) was collected through the heart and centrifugated with 4000rpm for 10 min, separating the serum and stored at -20℃. The rats were perfused immediately with 200ml normal saline from the heart to take out the brain completely, then cut olfactory bulb and prefrontal brain tissue. The 500mg brain tissue of ischemia area was cut backward from the optic chiasm (Bregma 0.00mm), put into a precooling mortar with cell lysis buffer according to the proportion of 1:4 (500μl lysis buffer + 5μl PMSF, No. P0013, Beyotime Institute of Biotechnology) to grind into the powder, then homogenized with ultrasonic waves, centrifuged

(Eppendorf, type:5801, Germany) for 10min at 12000r/min 4℃, eventually separating the supernatant. Finally, the concentration of the protein was assayed by BCA method, and stored at -20℃.

Detection indexes

The concentrations of MDA, NO and H_2O_2 in the homogenate of serum or brain tissue were respectively determined by thiobarbituric acid assay, nitratase reductase assay and chemiluminescence immunoassay. The homogenate of serum or brain tissue was redissolved in a room temperature and centrifuged to separate 100μl supernatant, then operating according to the kit instructions (Nanjing Jiancheng Bioengineering Institute). The values of absorbance were respectively determined at wavelengths of 550nm (MDA), 550nm（NO）and 405nm（H_2O_2）with the ultraviolet spectrophotometer (Beckmann DU640, USA) to calculate the concentrations of MDA (mmol/L), NO(μmol/L) and H_2O_2(mmol/L).

Statistical analysis

SPSS 17.0 software was used for data statistical analysis. Two-group comparison was made by t-test and data were expressed as mean ± standard error ($\bar{x} \pm s$) (Table 2), and multi-group comparison was made by analysis of variance (ANOVA) and the least significant difference (LSD) method for pairwise comparisons whether different level of administrating time and therapeutic dose had significant deviation or not, and whether their interaction on each detected index had significant deviation or not, meanwhile to explore the best therapeutic drug dose and time window (Table 3). Values were considered to be significant when *P* was less than 0.05.

RESULTS

Detection results

The concentrations of MDA, NO and H_2O_2 in serum and brain tissue in the model group rats were increased significantly compared with those in the sham group (t=15.22-35.77, P<0.01). And in the treatment group, the concentrations of MDA, NO and H_2O_2 in serum and brain tissue were obviously lower than those in the model group (t=4.60-12.76, P<0.01) (Table 2). It is suggested that the concentrations of each index in serum and brain tissue increse significantly after establishing the animal models and reduce after treated by picroside Ⅱ, and picroside Ⅱ can protect brain tissue from oxidative injury.

Analysis of variance (ANOVA) of the MDA content

The results showed that the MDA content in serum and brain tissue expressed at different degrees after treated by picroside Ⅱ (Table 3). According to the data statistical analysis with software of SPSS 17.0 (set α=0.05), the result of ANOVA showed that the significance probability of medication time (factor A) in serum and brain tissue was *P*<0.01, which indicated that the medication time of picroside Ⅱ could significantly influence on MDA expression (Table 4). The significance probability of interaction of independent variables (factor C) in serum and brain tissue was *P* > 0.05, so there was no interaction between medication time and therapeutic dose. The significance probability of therapeutic dose (factor B) in serum and brain tissue was *P*>0.05, and there was no significant deviation between different levels of the factor B. All data of each index were analyzed by the least significant difference (LSD) method for pairwise comparisons and the results showed that there was significant deviation of the MDA content in serum and brain tissue between administering drug at ischemia 1.0h (A1) and 2.5h (A4), and between administering drug at ischemia 1.5h (A2) and 2.5h (A4) (*P*<0.05), while no significant deviation between the rest combination and between each other of therapeutic dose (*P*>0.05). Considering the minimization of medication dose and maximization of therapeutic time, it was presumed that A2B2 is the best combination, namely the best therapeutic time window and the best therapeutic dose of picroside II for cerebral ischemia injury was ischemia 1.5 h with 10 mg/kg body.

ANOVA of the NO content

After treated by picroside Ⅱ, the results showed that the expression of NO in serum and brain tissue was at different degrees (Table 3). The significance probability of medication time in serum was smaller than 0.01 (*P*<0.01), while there was no significant deviation in brain tissue(*P*>0.05), which suggested that the medication time of picroside Ⅱ could significantly influence on MDA expression in serum (Table 5). There was no significantly influence on the NO content in serum and brain tissue between different levels of therapeutic dose and interaction of independent variables (*P*<0.05).The results of LSD showed that there was significant deviation of the MDA content in serum and brain tissue between administering drug at ischemia 1.0h (A1) and 2.5h (A4), and between administering drug at ischemia 1.5h (A2) and 2.5h (A4) (*P*<0.05), while no significant deviation between the rest combination and between each other of therapeutic dose (*P*>0.05).The results of LSD showed that there was significant deviation of the NO content between administering drug at ischemia 1.0h (A1) and 2.5h (A4), ischemia 1.5h (A2) and 2.5h (A4), also

Table 4. ANOVA of the MDA content

Variation source	SS_{Serum}	df	MS	*F*	*P*	SS_{Brain}	df	MS	*F*	*P*
Ischemia time	6.37	3	2.12	10.20	0.01	9.71	3	3.24	12.60	0.01
Drug dose	1.17	3	0.39	1.87	0.24	1.21	3	0.40	1.57	0.29
Time × Dose	0.27	3	0.09	0.43	0.74	2.57	3	0.86	3.33	0.10
Error	1.25	6	0.21			1.54	6	0.26		

Table 5. ANOVA of the NO content

Variation source	SS_{Serum}	df	MS	F	P	SS_{Brain}	df	MS	F	P
Ischemia time	614.62	3	204.87	9.29	0.01	2.50	3	0.83	4.54	0.06
Drug dose	267.92	3	89.31	4.05	0.07	1.25	3	0.42	2.28	0.18
Time×Dose	173.06	3	57.69	2.62	0.15	1.54	3	0.51	2.79	0.13
Error	132.33	6	22.06			1.10	6	0.18		

Table 6. ANOVA of the H_2O_2 content

Variation source	SS_{Serum}	df	MS	F	P	SS_{Brain}	df	MS	F	P
Ischemia time	23.21	3	7.74	15.22	0.01	22.29	3	7.431	15.26	0.01
Drug dose	5.60	3	1.87	3.67	0.08	8.71	3	2.903	5.96	0.03
Time×Dose	0.58	3	0.19	0.38	0.77	3.36	3	1.120	2.30	0.18
Error	3.05	6	0.51			2.92	6	0.487		

ischemia 2.0h (A3) and 2.5h (A4) in serum and between ischemia 1.0h (A1) and 1.5h (A2), ischemia 1.5h (A2) and 2.5h (A4) in brain tissue($P<0.05$), and no significant deviation between the rest combination in serum or brain tissue ($P>0.05$). There was significant deviation between therapeutic dose 5mg/kg (B1) and 20mg/kg (B3) in serum ($P<0.05$), and no significant deviation between the rest combination in serum or brain tissue ($P>0.05$). Considering the minimization of medication dose and maximization of therapeutic time, it was presumed that A2B3 is the best combination, namely the best therapeutic time window and the best therapeutic dose of picroside II was ischemia 1.5 h with 20 mg/kg body.

ANOVA of the H_2O_2 content

After treated by picroside Ⅱ, all rats showed the expression of H2O in serum and brain tissue at different degrees (Table 3). The significance probability of medication time in serum and brain tissue was $P<0.01$, which proved that the medication time of picroside Ⅱ could significantly influence on H_2O expression (Table 6). The significance probability of therapeutic dose and interaction of independent variables in serum and brain tissue were $P > 0.05$, so there was no significant deviation between different levels of therapeutic dose and no interaction between medication time and therapeutic dose. The results of LSD showed that there was no significant deviation of the H_2O_2 content between ischemia 1.0h (A1) and 1.5h (A2), and ischemia 1.0h (A1) and 2.0h (A3) in serum and between ischemia 1.0h (A1) and 2.0h (A3) in brain tissue ($P>0.05$), but a significant deviation existed between the rest combination in serum and brain tissue ($P<0.05$). There was significant deviation between therapeutic dose 5mg/kg (B1) and 10mg/kg (B2) ($P<0.05$), and no significant deviation between the rest combination in serum ($P>0.05$). There was no significant deviation ($P>0.05$) between therapeutic dose 5mg/kg (B1) and 40mg/kg (B4), also 10mg/kg (B2) and 20mg/kg (B3), while a significant deviation found between the rest combination in brain tissue ($P<0.05$). Considering the minimization of medication dose and maximization of therapeutic time, it was presumed that A2B2 (in serum) and A2B3 (in brain tissue) are the best combination, namely the best therapeutic time window and the best therapeutic dose of picroside II were ischemia 1.5 h with 10 mg/kg body in serum and ischemia 1.5 h with 20 mg/kg body in brain tissue.

DISCUSSION

Orthogonal layout can balance sampling in the changing range of variable factors, and can enhance the representation of each test with minimum animal number and test times. Orthogonal layout has characteristics of balanced scattering, which satisfies some prerequisites of a comprehensive test, shortened test cycle and elevated

test efficiency to achieve a better test aim. In this paper, the authors applied orthogonal layout to design roundly, compared synthetically with statistical analysis to obtain a better therapeutic schedule to get the best treatment effectiveness with a small number of tests.

Recently, some studies have found that the active components of the traditional Chinese medicine ginkgolide B (Huang et al., 2008) and Salvia miltiorrhiza Bungefalba (Zhang et al., 2010) can significantly reduce the content of MDA, delay the activity of GSHPx down, lessen the mitochondrial injury induced by cerebral ischemia reperfusion in rats, inhibit apoptosis and narrow the scope of cerebral infarction. The liquiritin can significantly reduce the concentration of MDA in serum and raise the activity of CAT and GSHPx to improve the neurobehavioral function of mice (Sun et al., 2010). The salidroside (Yan et al., 2008) can significantly lessen the content of NO after cerebral ischemia reperfusion in rats. The chrysophanol and emodin (Wang et al., 2012) can significantly decrease the content of H_2O_2 in brain tissue in cerebral ischemia reperfusion mice and improve the activity of CAT in brain tissue to play a protective effect on cerebral ischemia reperfusion injury. Picrorrhiza scrophulariflora belongs to the traditional Chinese medicine, and its principal active components are compound of iridoid glycosides having effects of anti-inflammatory and anti-oxidant (Guo et al., 2010). Tao YW found that picroside Ⅱ had a significant protective effect on the injury of PC12 induced by hydrogen per oxide (H_2O_2) (Tao et al., 2003). Guo MC (Guo et al., 2007) established oxidative stress models of PC12 cells with glutamic acid and proved that picroside Ⅱ can inhibit the generation of free radicals through improving the activity of antioxidase in cells to play a role of anti-lipid peroxidative injury. Gu W (Gu et al., 2008) found that picroside Ⅱ had a protective effect on the L-02 cell injury caused by oxidative stress, and its mechanism may be related to the reduction of the reactive oxygen species content in cells and the inhibition for the mitochondrial membrane potential down.

In this experiment, the content of MDA, NO and H_2O_2 can directly reflect the changes of the free radicals content in vivo in cerebral ischemic injury. The content of MDA, NO and H_2O_2 increased significantly in serum and brain tissue after establishing the animal models and reduced after injecting picroside Ⅱ intraperitoneally. It was suggested that picroside Ⅱ can enhance the activity of endogenous antioxidant enzymes and clear the excess oxygen radicals to protect brain tissue from oxidative injury. According to the orthogonal experimental design of [$L_{16}(4^5)$], the results showed that there was a significant deviation in treatment effectiveness between ischemia time and therapeutic dose of picroside Ⅱ and the best combination was not with accordant according to the different indexes. Considering the minimization of medication dose and maximization of therapeutic time, it is presumed that A2B2 and A2B3 are the best combinations, namely the best therapeutic time window and the best therapeutic dose of picroside II is ischemia 1.5 h with 10~20 mg/kg body. The mechanism of cerebral ischemic injury is very complicated and only six indexes were observed in this experiment, so some golden evaluating indexes need to be further studied in further experiments to explore the certain effect and mechanism and the best therapeutic time window and the best therapeutic dose of picroside II.

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REFERENCES

Amemiya S, Kamiya T, Nito C, Inaba T, Kato K, Ueda M, Shimazaki K, Katayama Y (2005). Anti-apoptotic and neuroprotective effects of edaravone following transient focal ischemia in rats. Eur J Pharmacol. 516(2): 125-130.

Asano H, Horinouchi T, Mai Y, Sawada O, Fujii S, Nishiya T, Minami M, Katayama T, Iwanaga T, Terada K, Miwa S (2012). Nicotine- and tar-free cigarette smoke induces cell damage through reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase. J Pharmacol Sci. 118(2):275-87.

Brown GC (2010). Nitric oxide and neuronal death. Nitric Oxide. 23(3):153-165.

Cejas P, Casado E, Belda-Iniesta C, De Castro J, Espinosa E, Redondo A, Sereno M, García-Cabezas MA, Vara JA, Domínguez-Cáceres A, Perona R, González-Barón M (2004). Implieations of oxidative stress and cell membrane lipid peroxidation in human cancer (Spain). Cancer Cuases Contor. 15(7):707-719.

Chen S, Liang H (2004). Changes of Serum sICAM-1 and MDA in Patients with Acute Intracerebral Hemorrhage. Chin J Integr Med Cardio-/Cerebrovas Dis. 2(3):148-151.

Choi DW (1988). Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1(8):623-634.

Gu W, Fan XJ, Wu J, Niu ZQ (2008). Protective effect of Picroside Ⅱ against H2O2-induced damage in L-02 cells. World Chinese J. Digestol. 16(29):3274-3278.

Guo MC, Cao Y, Liu JW (2007). Protective effects of picroside Ⅱ on glutamate injury of PC12 cells. Chinese J. Clinical Pharmacol. and Therapeutics. 12(4):440-443.

Guo YL, Xu XY, Li Q, Li Z, Du F (2010). Anti-inflammation effects of picroside Ⅱ in cerebral ischemic injury rats. Behavioral Brain Functions. 6:43-49.

Huang JY, Sun JN, Mei SC, Huang JM (2008). Protective effects of ginkgolide B on cerebral ischemia reperfusion injury in rats. Chinese Pharmacological Bulletin. 24(2):269-272.

Ito Y, Ohkubo T, Asano Y, Hattori K, Shimazu T, Yamazato M, Nagoya H, Kato Y, Araki N (2010). Nitric oxide production during cerebral ischemia and reperfusion in eNOS and nNOS-knockout mice. J Curr Neurovasc Res. 7(1):23-31.

Li M, Zhao J, Hu Y, Lu H, Guo J (2010a). Oxygen free radicals regulate energy metabolism via AMPK pathway following cerebral ischemia. Neurol Res. 32(7):779-784.

Li P, Matsunaga K, Yamakuni T, Ohizumi Y (2002). Picrosides Ⅰ and Ⅱ, selective enhancers of the mitogen-activated protein kinase-dependent signaling pathway in the action of neuritogenic substances on PC12D cells. Life Sci. 71(15):1821-1835.

Li Q, Li Z, Xu XY, Guo YL, Du F (2010b). Neuroprotective properties of picroside II in rat model of focal cerebral ischemia. Int J Mol Sci.

11(11):4580-4590.
Li Z, Xu XY, Li Q, Zhang MZ (2010a,b), Shen W. Protective mechanisms of picroside Ⅱ on aquaporin-4 expression in a rat model of cerebral ischemia/reperfusion injury. Neural Regen Res. 5(6):411-417.
Liu Y, Li C P, Zhu L (2008). Oxidative Stress, Lipid peroxidation and liver cell apoptosis play an important role in non-alcoholic fatty liver disease. Med Recap. 14(10):1468-1470.
Lv SX, Jia HL, Chen CG, Sun LH, Xu CQ (2011). The protective effect of propyl gallate injection on acute cerebral ischemic reperfusion injury. Journal of Apoplexy and Nervous Diseases. 28(10):935-937.
Márquez-Martín A, Jiménez-Altayó F, Dantas AP, Caracuel L, Planas AM, Vila E (2012). Middle cerebral artery alterations in a rat chronic hypoperfusion model. J Appl Physiol. 112(3):511-518.
Pei HT, Su X, Zhao L, Li HY, Guo YL, Zhang MZ, Xin H (2012). Primary study for the therapeutic dose and Time window of picroside Ⅱ in treating cerebral ischemic injury in rats. Int J Mol Sci. 13(3):2551-2562.
Perluigi M, Di Domenico F, Giorgi A, Schininà ME, Coccia R, Cini C, Bellia F, Cambria MT, Cornelius C, Butterfield DA, Calabrese V (2010). Redox proteomics in aging rat brain: involvement of mitochondrial reduced glutathione status and mitochondrial protein oxidationin the aging process. Neurosci Res. 88(16):3498-3507.
Rey B, Roussel D, Teulier L, Eyenga P, Degletagne C, Belouze M, Duchamp C (2011). Functional argument for the existence of an avian nitric oxide synthase in muscle mltochondria: effect of cold acclimation. FEBS Lett. 585(1):173-177.
Shibata N, Kobayashi M (2008). The role for oxidative stress in neurodegenerative diseases. Brain Nerve. 60(2):157-170.
Sun YX, Tang Y, Wu AL, Liu T, Dai XL, Zheng QS, Wang ZB (2010). Neuroprotective effect of liquiritin against focal cerebral ischemia/reperfusion in mice via its antioxidant and antiapoptosis properties. J Asian Nat Prod Res. 12(12):1051-1060.
Tao YW, Liu JW, Wei DZ, Su W, Zhou WY (2003). Protective effect of picroside-Ⅱ on the damage of cultured PC12 cells in vitro. Chinese J. Clin. Pharmacol. Therapeutics. 8(1):27-30.
Tomatsuri N, Yoshida N, Takagi T, Katada K, Isozaki Y, Imamoto E, Uchiyama K, Kokura S, Ichikawa H, Naito Y, Okanoue T, Yoshikawa T (2004). Edavavone, a newly developed radical scavenger protects against ischemia-reperfusion injury of the small intestine in rats. Int J Mol Med. 13(1):105-109.
Wang S, Zhang DS, Zhang L, Zhang HH, Xue GP (2012). Effects of Emodin on hydrogen peroxide and catalase with cerebral ischemia and reperfusion in mice. Chin J Pharmacol Toxicol. 26(3):417.
Wang X, Shuaib A (2007). Neuroprotective effects of free radical scavengers in stroke. Drugs Aging. 24(7): 537-546.
Xi H, Akishita M, Nagai K, Yu W, Hasegawa H, Eto M, Kozaki K, Toba K (2007). Potent free radical scavenger, edaravone, suppresses oxidative stress-induced endothelial damage and early atherosclerosis. Aherosclerosis. 191(2):281-289.
Yan TH, Jia Y, Yang W, Wang QJ (2008). Protective effects of on focal cerebral is chemic-reperfusion injury in rats. Chinese Pharmacological Bulletin. 24(11):1521-1524.
Yuan Y, Yang JQ, Zhou QX (2011). Relationship between SOD2 expression and free radical level in neurodegeneration induced by ischemia-reperfusion in mouse. Basic Clin Med. 31(11):1229-1233.
Zhang QL, Sun YB, Wang HY, Song SJ, Bai B (2010). Effects of Salvia miltiorrhiza Bunge.f.alba on mitochondrial damage and apoptosis induced by cerebral ischemia and reperfusion. Chin J Pathophysiol. 26(4): 725-729.