Animal drugs in treatment of cerebral ischemia and their mechanisms

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Abstract:

Over the past half century, toxins or preparations from animals have drawn great attentions for their significant therapeutic effects in treatments of cerebral ischemia. Here, we review several such animal drugs, their mechanism of actions, and its outlook.

Keywords: Ischemia, animal drug, mechanism, review

Cerebrovascular disease has become one of the most frequent causes of human death. It could be classified into two major categories, ischemic and hemorrhagic. About 1.5 million people suffer one or more occurrences of strokes in China each year, which accounts for about one million deaths [1]. Among the causes surveyed in China, 44.6% of the cases were due to cerebral hemorrhage, and about 46.4 % was because of cerebral thrombosis [2]. In other countries, ischemic stroke has also been found to be more common, representing 59.2% to 85% of the cases. With an exception for Japan, cerebral hemorrhage cases are generally below 20%.

Based on the different underlying pathogeneses of ischemic cerebrovascular diseases, their treatments vary accordingly, and usually include thrombolytic agents, anti-coagulants, anti-platelet agents, and prevention therapies. Commonly used drugs are alteplase, aspirin, heparin, and other biologics or small molecules.

As an alternative medicine, animal drugs have drawn great interests in the past half century, mainly due to their significant therapeutic effects observed in multiple disease area including cerebral vascular diseases. Commonly used animal drugs are toxins and preparations from animals such as leeches, snakes, earthworms and others. Here, we review several such animal drugs and their mechanism of actions.

1. Pathogenesis of cerebral ischemia

Cerebral ischemia often leads to severe brain damages due to energy depletion, and accumulations of toxic molecules such as excitatory amino acids, reactive oxygen species, inflammatory cytokines, nitro oxide and intracellular calcium. Animal drugs usually achieve cerebral protections by targeting multiple pathogenesis simultaneously. Among the many mechanisms are the capabilities to reduce excitatory neurotransmitter, decrease acidic toxicity, inhibit lipid peroxidation and nitration, reduce inflammation, and inhibit cellular apoptosis.

2. Animal toxin and extracts in treatment of cerebral ischemia and their mechanism

2.1 Snake venom preparations

Snake venom is the saliva secreted from the venom glands of poisonous snakes such as agkistrodon acutus and cobra. Toxic proteins represent about 90 % to 95% of the dry weight of snake venom [3]. In China, several snake venom based medicines have been approved to treat cerebral vascular diseases, which include defibrase, SVATE and batroxobin.

2.1.1 Defibrase

In clinical, defibrase has been used for the treatment of thromboembolic disease, such as cerebral thrombosis, limb venous thrombosis and retinal vein thrombosis. It was first separated and purified from acutus venom in 1978 [4]. It is able to dissolve fibrinogen and fibrin in the plasma to disintegrate clot already formed. It also decreases the blood viscosity and prolongs the coagulation time.

2.1.2 SVATE (Ahylysantinfarctase)

SVATE is a purified fraction of snake venom showing antithrombin effects. It is a mixture of multiple enzymes, including arginase, phosphodiesterase and other proteolytic enzymes. It demonstrated relatively good effects on ischemic cerebrovascular disease in clinic with few side effects [5, 6]. Pharmacological studies have confirmed the anticoagulant and thrombolytic effects of ahylysantinfarctase, and demonstrated that it improved microcirculation and enhanced recoveries of nerve cells from injuries [7]. SVATE is one of the commonly used venom preparations in clinic in China.

2.1.3 Batroxobin

Batroxobin, introduced into China from Japan in 1994, has become one of the eight products being used in the hospitals for treatment of thrombosis. As a defibrase, batroxobin is able to significantly lower fibrinogen levels in the plasma. It can also inhibit platelet aggregation [9]. It was reported that batroxobin reduced neuronal damages and effectively improved the neurological defects in patients that suffered acute cerebral infarction. It is not clear if batroxobin is able to decrease the plasma level of lysophosphatidic acid, which is thought to be a molecular biomarker for platelet activation and its elevation is often a warning sign for abnormal thrombosis [9].

2.2 Leech preparations

In China, leeches was recorded as an animal medicine as early as in "Shen Nong's Herbal Classic”. Purification of hirudin from the salivary gland of leech was achieved in 1950s [10]. In 1980s, recombinant hirudin was generated thanks to the advancement in molecular biology and genetic engineering. Numerous clinical trials have demonstrated that hirudin is more effective than heparin in preventing formation of vein thrombosis [10]. Pharmacology studies have confirmed that hirudin has anticoagulant effects, and demonstrated that it could prevent platelet aggregation and improve microcirculation.

2.3 Earthworm preparations

Di Long (Chinese name for earthworm) is a general nomenclature of dried *Pheretima aspergillum* (E. Perrier), *P. vulgaris* (Chen), and *P. guillemi* (Michaelsen). It belongs to the genus of Pheretima, family of Megascolecidae, and phylum of Annelida. It is an important animal ingredient originally recorded in the "Shen Nong's Herbal Classic". Possessing a characteristic of Salty and Cold, this traditional Chinese medicine (TCM) drug owns therapeutic efficacies such as diuretic, analgesic, relieving asthma, bringing down high blood pressure, antipyretic, and anticonvulsant effects. In another ancient Chinese Pharmacopoeia "Compendium of Materia Medica (Ben Cao Gang Mu)", it was described that this animal drug displayed important therapeutic effects such as "dredging and activating the meridian", "promoting blood circulation for removing blood stasis", as well as roles in prevention and treatment of cardiovascular and cerebrovascular diseases.

In 1982, lumbrokinase was first purified from earthworm preparations and demonstrated fibrinolytic activity [11]. Since then, active ingredients in earthworms have been studied extensively around the world for treatment of ischemic stroke.

Earthworm preparations have been shown having the following pharmacological effects. (1) Fibrinolysis effect. In rabbits, earthworm extracts significantly reduced fibrinogen levels in plasma and prolonged euglobulin lysis [12]. (2) Anticoagulant effects. In mice, earthworm extract was reported to delay clotting significantly [13]. (3) Prevention of platelet aggregation. Earthworm extract significantly reduced the rate of platelet adhesion, inhibited platelet aggregation, reduced blood clots, and dissolved thrombus [14]. In rats it was able to increase cerebral blood flow and decrease cerebral vascular resistance. (4) Protective effects on cerebral blood vessels. It was demonstrated that streptokinase in earthworms could inhibit the formation of thrombus following cerebral ischemia in rabbit and reduce the damages to cerebral tissues in rats [15].

2.4 Scorpion preparations

The custom dried bodies of *Buthus martensii* Karsch (Buthidae) have been recorded to possess anticonvulsant, antiepileptic, analgesic, cardiovascular protection, anti-tumor and other pharmacological effects. Active peptides have been purified from Scorpion venom which showed fibrinolytic effects and prevented injuries from cerebral ischemia-reperfusion [16]. It was reported that these peptides significantly improved neurological functions after MCAO and reduced the infarct volume in rats ($P < 0.05$) [17]. These protective effects were thought to be attributed to reduced expression levels of inflammatory cytokines in serum such as TNF-α, IL-6 and IL-8.

In recent years, Chinese scientists have started to use scorpion containing compositions to treat cerebrovascular disease. Tong Xin Luo capsule is such a composition that contains scorpion as a main drug. In Zea-Longa ischemia-reperfusion models, Tong Xin Luo capsule at 20 mg/kg induced a significant decrease in neurological damage, a decrease in brain edema and a reduction in infarct size [18]. Such treatment also improved mitochondrial function, inhibited the downregulation of SOD level and upregulation of MDA in homogenate of brain tissues. These changes may underlie the mechanism of the protective effects of scorpions on cerebral ischemia-reperfusion. In a focal cerebral ischemia rat model, Tong Xin Luo capsule had protective effects on injuries induced by blocking the blood flow in the middle cerebral artery for 2 h and then reperfused for 72 h [19]. These effects may be related to an upregulation of IL-TGF- β1 protein. In a modified Pulsineli 4 - vessel occlusion (4-VO) model of cerebral ischemia, Shenxiong Huayu Capsule, another scorpion containing composition, was able to inhibit accumulation of Aβ peptides in hippocampus region as indicated by immunostaining and reduced apoptosis of nerve cells by tunnel assays [20].

2.5 Gadfly preparations (Tabanus)

This animal drug is composed of female dried bodies with various species of Tabanidae insects such as *Atylotus bivittateinus* Takahasi, *Tabanus mandarinus* Schiner, *Tabanus bivittatus* Matsumura, *Tabanus pleskei* Krober, and *Tabanus budde* Portschinskg, etc. It is a commonly-used clinical TCM for "promoting blood circulation for removing blood stasis". "Shen Nong's Herbal Classic" recorded that insects of this genus showed properties of Bitter and slightly Cold, which was used for removing blood stasis, improving stuffiness and rigidity, diminishing abnormal mass, clearing chills and fever, and promoting blood circulation and nine orifices.

It was reported that gadfly preparations has weak anti-thrombin effects *in vitro* and it can induce activation of the fibrinolytic system both *in vitro* and *in vivo*. For example, its thrombolytic effect was demonstrated in an *in vitro* thrombolysis study [21]. Polysaccharides in Hybomitra erberi (Brauer) gadfly was able to significantly prolong the clotting time, reduce the exogenous coagulation system factor activity and increase the vitality of the fibrinolytic system to prevent thrombosis [22]. At high doses genuine gadfly extracts could significantly prolong bleeding time, reduce plasma fibrinogen levels and inhibit platelet aggregation in rats [23]. In addition,

gadfly and leeches have been combined to treat cerebral vascular disease and showed significant therapeutic effects. Its mechanism of action may be related to modification of blood rheology [24].

2.6 Eupolyphaga (ground beetle) preparations

This animal drug involves female dried bodies of two common species of *Eupolyphaga sinensis* Walker, and *Polyphaga plancyi* Boleny or *Steleophaga plancyi* Boleny, which are reported to possess thrombolysis ability. Aqueous extract of Eupolyphaga at 1 mg/kg significantly enhanced the endurance of rabbit under severe hypoxia situation to maintain normal heart function [25]. Eupolyphaga significantly prolonged the survival time of mice with both bilateral carotid artery and vagus nerve ligated [26]. After cerebral ischemia and reperfusion, there was an enhancement of SOD activity in brain tissue, reduction in NOS activity, an increase in GSH content, and decreases in levels of NO and MDA. This suggested that antioxidant effects and inhibition of free NO may explain the protective effects of Eupolyphaga extract on cerebral ischemia and reperfusion in mice. Using arteriovenous bypass thrombosis assay, Eupolyphaga aqueous extract was found to have anticoagulation and thrombolysis effects. In that study, Eupolyphaga significantly reduced the weights of experimental thrombosis in rats, significantly prolonged prothrombin and clotting time *in vitro* [27]. Eupolyphaga was able to induce fibrinolysis, not anticoagulant, during its treatment of various vascular diseases [28]. These studies suggest that Eupolyphaga has great potentials in prevention and treatment of thrombotic cardiovascular disease.

2.7 Spider preparations

Spider **(**arachnid) is a generic term for all species belonging to Araneida, Arachnida, Arthropoda. Spider toxins contain a variety of ingredients and have been widely studied in neurobiology, molecular pharmacology and molecular toxicology [29, 30]. Spider toxin based drug has demonstrated unmatched efficacy in treating stroke and epilepsy [31]. In a rat ischemia-reperfusion injury model established by a combination of modified four-vessel occlusion and subarachnoid drainage, it was found that drug treatment groups had significant lower RNA and protein levels of Fas, FasL and FADD [32]. Subarachnoid administration of Tiger spider toxin - I (HWTX-I) protected rats from cerebral ischemia-reperfusion injury, which may be due to inhibition of FAS induced apoptosis.

2.8 Centipede preparations

Centipede is the dried bodies of *Scolopendra subspinipes mutilans* L. Koch. Modern pharmacological studies demonstrated that centipede has anticoagulant and thrombolytic effects, which may be related to the proteases, hemolytic factors and other blood-activating factors present in the venom [33]. In an atherosclerosis (AS) rabbit model established through high-fat feeding method, it was demonstrated that centipede treatments prevented cell aggregation and improved blood rheology through reducing lipoprotein and fibrinogen levels in the plasma and decreasing the low shear rate and viscosity of whole blood [34]. Centipedes were able to decrease the number of red blood cells (RBC) and the concentration of hemoglobin, reduced the trend of RBC hematocrit and prolonged the clotting time [35]. It was also shown that centipedes significantly increased the diameters and numbers of open capillaries that led to anticoagulant effects. *Scolopendra subspinipes mutilans* L. Koch fibrinolytic enzyme (SSFE) was isolated from Scolopendra centipede using ion exchange chromatography and molecular sieve preparation methods, and was demonstrated having antithrombotic effects [36]. In a middle cerebral artery focal cerebral ischemia-reperfusion rat model established through suture, centipede extracts could decrease the levels of vWF and TPO in the plasma, improve injury caused by cerebral ischemia-reperfusion, reduce the damages to endothelial cells and platelet function, effectively inhibit platelet adhesion and aggregation, prevent thrombosis, thereby reduce the brain damage [37]. Thus, it could be used for prevention and treatment of cardiovascular and cerebrovascular disease pathways. It was also reported that some activities of centipede toxin could be inhibited by o-phenanthroline, indicating the involvement of metalloprotease enzymes [38].

2.9 Jiangcan preparations

Jiangcan is the dried body of 4-5 instar larvae of *Bombyx mori* L. infected and killed by *Beauveria bassiana* (Bals.) Vuill. Modern pharmacological studies have shown that Jiangcan has anticoagulant and thrombolytic effects. In a Beyers' venous thrombosis animal model, intravenous injection of Jiangcan extracts induced a significant reduction in the weight of thrombosis formed ($P < 0.01$), a decrease in the levels of plasminogen and fibrinogen, a decrease in the euglobulin lysis time ($P < 0.05$), but a prolongation of activated partial thromboplastin time (KPTT), prothrombin time (PT), thrombin time (TT) ($P < 0.01$) [39]. This suggested that Jiansan has pro-fibrinolytic and anticoagulant activities and could be used for thrombotic diseases. It was found Jiangcan could inhibit the formation of thrombus through activation of the fibrinolytic system [40].

2.10 Buffalo horn preparations

Buffalo horn is the horn of *Bubalus bubalis* Linnaeus. In treatment of cerebral ischemic disease, raw buffalo horn is often combined with other herbs/medicine. One example is Qingre Huayu decoction used in several pharmacological studies. Using ischemia–reperfusion rat models, it was observed that mRNA and protein levels of glial cell line-derived neurotrophic factor (GDNF) were significantly increased in the cerebral ischemic penumbra about 3 hours after treatment with Qingre Huayu decoction, peaked around 6 h, started to weaken at 12 h and reduced to normal levels three days later [41]. This suggested that buffalo horn can promote the expression of GDNF after cerebral ischemia to protect neurons.

In another report, the authors established intraluminal middle cerebral artery occlusion (MCAO) rat models and measured the degree of neurological deficit, as well as pathological changes in the brain tissues by hematoxylin and eosin staining [42]. Rats were divided into control group, sham surgery group and surgery group, and for each group they were treated with high, medium and low doses of Qingre Huayu decoction. The results revealed that rats treated with middle and high doses of drugs showed less damages in nerve functions, which correlated to the pathological changes in brain tissues, suggesting protection effects of the drug to focal cerebral ischemia-reperfusion injury in ischemic cerebrovascular disease.

2.11 Moschus preparations

Moschus has been used for treatment of cerebral ischemia and encephalitis. It is secreted from the glandular sac of male musk deer. Chemically it is mainly composed of macrocyclic ketones, with moschus ketone as the major physiologically active substance [43].

Rat focal cerebral ischemia and reperfusion injury model has been used to study the effects of moschus on neurology, and measured multiple parameters such as brain water content, the ratio of brain body, histopathology, and blood-brain barrier permeability [44]. It was found that moschus ketone exerted protective effects against cerebral ischemia brain edema, enhanced blood-brain barrier permeability, and increased the ratio of brain body and brain water content in rats. Mechanistic studies showed that moschus ketone induced excitements in the sympathetic nervous system, which further stimulated β receptor in the brain arteries, lowered the cerebral vascular tone, improved flexibility and vasodilatation of blood vessels that prompted cerebral blood flow, enhanced metabolism of oxygen and energy in the brain, thereby protecting neurons from cerebral ischemia injury.

In addition, combinations of moschus with other agents demonstrated protective effects on cerebral ischemia. For example, in a modified intraluminal focal cerebral ischemia 2/24 h of reperfusion injury model, the effect of moschus was dertermined on rats neurological function, infarct size and blood-brain barrier damage through 2,3,5-triphenyltetrazolium chloride staining [45]. The results showed that moschus can reduce cerebral ischemia - reperfusion infarct volume, reduce symptoms of nerve damage, lower serum IL-6, IL-1β levels and brain tissue myeloperoxidase (MPO), nitrous oxide synthase (NOS) activity. The mechanism may be related with the release

of inflammatory mediators. Moschus has been combined with borneol to treat ischemic diseases. For example, It was shown in a focal cerebral ischemia-reperfusion injury rat model that the combination of moschus and Borneol reduced the focal cerebral ischemia-reperfusion infarct volume, improved neurobehavioral symptoms after cerebral ischemia, and reduced brain water content and the blood-brain barrier permeability. This indicated a protective role in the focal cerebral ischemia-reperfusion injury in rats [46].

3. Other animal drugs in treatment of cerebral ischemia [47]

According to a report from a group in National University of Mexico, saliva from bats was able to dissolve blood clots, and was used to treat patients with myocardial infarction and cerebral thrombosis with fewer side effects [48]. It was thought that some thrombolytic plasminogen contributed to the effects. This drug is expected to be on the market in 2-4 years [48]. Bee Venom is an acidic and transparent liquid and contained element like carbon, hydrogen, sulfur, phosphorus, calcium, chlorine, nitrogen and others. Bee venom was reported to have hemolytic and anti-clotting effects. At therapeutic doses, it rarely induced hemolytic reaction [49]. At higher doses, it prolonged blood clotting time both *in vitro* and *in vivo*. It was also able to reduce thromboxane, which help relieve the symptom of rheumatoid arthritis [50].

4 Outlook of animal Drugs in treatments of cerebral ischemia

China is enriched with animal resources. In this review we described various animal drugs that are often used in treatment of cerebral ischemia disease. Our research team at the Key Laboratory of Medical Insects and Spiders Resources for Development and Utilization at Yunnan Province has also achieved good results in treatment of cerebral thrombosis using animal drugs [51].

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References

[1] Zhang, J.F.; Xu, Y.H.; Li, Z.Z.; Wang, H.L.; Wang, H.J.; Yin, J. Trends in epidemiological survey for awareness of risk factors of the Ischemic Stroke. J. Brain Nerv. Dis., 2007, 15, 418-420.

[2] Hong, Z. The epidemiology of stroke and its risk factors. Chin. J. Stroke, 2006, 7, 559-563.

[3] Chen, N.; Fu, X.-Y.; Jiang, W.-J., Zhao, S.-J. Classification and application of proteins from snake venom. Chin. J. New Drugs, 2009, 18, 1204-¬¬1210. .

[4] Zhang, H.J.; Xiao, C.H.; Tang, S.Z.; Yang, J.R. Study on isolation from the venom of Agkistrodon acutus and its biological activity . Zoologic. Res., 1980, 1, 157-162.

[5] Chen, X.Y. In: New Pharmacology. 14th ed: People's Medical Publishing House: Beijing, 1998. pp. 395-396.

[6] Ma, T.L. The Venomous anti-thrombus enzyme (VATE) of ahylysantinfarctase for its effects on clinical observation of ischemic cerebrovascular disease. Aerospace Med., 2010, 21, 972-972.

[7] She, S.F. The clinical application of ahylysantinfarctase. J. Trad. Chin. Med. Uni. Hunan, 2011, 31, 78-80.

[8] Haserück, N.; Erl, W.; Pandey, D.; Tigyi, G.; Ohlmann, P.; Ravanat, C. The plaque lipidlysophosphatidic acid stimulates platelet activation and platelet-monocyte aggregate formation in whole blood: involvement of P2Y1 and P2Y12 receptors. Blood, 2004, 103, 2585-2592.

[9] Zhu, W.H.; Yu, J.; Ni, X.S. Influence of Batroxobin on plasma level of lipidlysophosphatidic acid in patients with acute cerebral infarction. J. Clin. Neurol., 2006, 19, 330-332.

[10] Prisco, D.; Falciani, M.; Antonucci, E., Gensini, G.F. Hirudins for prophylaxis and treatment of venous thromboembolism. Semin.Thromb. Hemost., 2001, 7, 543-549.

[11] Mihara, H.; Sumi, H.; Yoneta T.; Mizumoto, H.; Ikeda, R.; Seiki, M.;Maruyama, M. A novel fibrinolytic enzyme extracted from the earthworm, Lumbricus rubellus. Jpn. J. Physiol., 1991, 41, 461-472.

[12] Zhang, Z.X.; Zhong, L.W.; Zhen, G.P.; Wang, H.Z.; Zhao, J.B.; Shan, H.R.; Wang, F.F. Effects of crude extract of earthworm on promoting blood circulation to removing stasis. Chin. J. Integr. Trad. West. Med.,1992, 12, 741-743,710.

[13] He, H.; Che, Q.M.; Sun, Q.S. Anticoagulant effects of Pheretima extracts. Chin. Tradi. Herb. Drug, 2007, 27, 733-735.

[14] Yin, S.M.; Chu, Y.P.; Wu, P. Pharmacodynamic test of active extract of Lumbricus . Chin. Trad. Herb. Drugs, 2002, 33, 64-66.

[15] Yang, M.; Dong, G.H.; Zhang, F.Q.; Yang, Z.X.; Zhang, Y.H. Study on earthworm fibrinoly activity of Eisenia Foetida Sarigny-VI,Study on rabbits thrombusolysis and protection of rat brain deficit oxygen of earthworm fibronolysin. Biotechnology, 1995, 5, 9-11.

[16] Lu, X.R.; Bai, L.; Zhu, G.J.; Zhang, Y.; Duan, Y.P. Effect of Scorpion and Bee venom peptide(SBP) on fibrinolytic system in rat. Basic Clin. Med., 1994, 14, 68-70.

[17] Wang, Q.Y.; Wu, F.J.; Li, J.L. Effect of scorpion venom fibrinolytic active peptide on cerebral ischemia/reperfusion injury in rats. Chin. J. Modern Appl. Pharm., 2011, 28, 403-406.

[18] Ding, S.J.; Li, J.S.; Bi, X.Y.; Qiu, Y.Q. Protective effect and mechanism of Tongxinluo on cerebral ischemia reperfusion in SD rat model. Chin. J. Cerebrovasc. Dis.(Electronic Version), 2010, 4, 3-10.

[19] Zou, Y.M.; Cui, X.Q. Protective effect of Tongxinluo on focal cerebral ischemic injury in rat. China Association of Chinese Medicine, Chinese Association of the Integration of Traditional and Western Medicine, The Basic and Clinical Researches upon Collateral Diseases (5). 2009: 291-293.

[20] Li, J.M.; Zhao, Y.N.; Zhang, S.S.; Chen, C.X.; Ma, S.H.; Li, S.X. Protective effects of Shenxionghuayu capsule on cerebral ischemia injury in rats. Chin. J. Gerontol., 2011, 31, 3309-3311.

[21] Zhai, Y. Study on chemical constituents-Ⅰ from Tabanus bivittatus Mats. Masterate Dissertation of Shenyang Parmaceutical University: Shen yang, July 2007.

[22] Jin, W. Pharmacological study on Mangchong (Gadfly) in anticoagulant material. Informat. Trad. Chin. Med., 2000, 27, 64-66.

[23] Chen, Y.Y.; Meng, Q.D.; Tong, L.; Xu, J. Effect of Eupolyphaga sinesis Walker(ESW) water extract on bleeding time and fibrinogen and platelet aggregation. J. Southern Med. Uni., 1990, 10, 260-261.

[24] Chen, Z.G.; Zou, Y.H.; Liu, C.; Zhen, Y.W.; Dong, G.R.; Wang, S.T.; Dai, C.; Li, X.Q.; Deng, Z.M.; Long, C.X.; Han, X.J.; Fan, J.P.; Wang, J.X. Treatment of acute ischemic stroke with Shuxuetong injection. J. Beijing Uni. Trad. Chin. Med. (Clin.Med edn), 2005, 12, 5-8.

[25] Huang, J.B.; Feng, G.Z.; Liu, X.S.; Fan, Y.M.; Song X.L. Experimental research for the anti-cardio-cerebral anoxia in rabbit of Eupolyphaga sinesis Walker. J. Changzhi Med. College, 1994, 8, 102-104.

[26] W.H. He, R. Zeng, X.H. Cheng. The protective effect of Eupolyphaga sinesis Walker against cerebral ischemia-reperfusion injury. Pharmacol. Clin. Chin. Mater. Med., 2008, 24, 43-44.

[27] Zhou, C.F.; Lai, M.; Wang, X.H.; Gao, X.J. Study on the anti coagulation of from Eupolyphaga sinesis Walker. J. Changchun Uni. Trad. Chin. Med., 1999, 15, 47.

[28] Lang, J.; Zhen, Y.G.; Chen, L.W.; Zhang, R.P.; Chen, H.Z.; Li, J. Study of Eupolyphage Sinesis Walker on the anticoagulant and facilitated fibrinolysis. Pharmacol. Clin. Chin. Mater. Med., 2006, 22, 108-109.

[29] Pan, J.Y.; Hu, W.J.; Liang, S.P. Purification, sequencing and characterization of Hainan toxin-Ⅵ, a neurotoxin from the Chinese bird spider Selenocosmia hainana. Zoolog. Res., 2002, 33, 280-283.

[30] Xia, X.; Xiong, X.; Li, D.L.; Xiao, Y.C.; Wang, X.C.; Liang, S.P. Solid-phase synthesis and biological characterization of S12A-HNTX-IV and R29A-HNTX-IV: two mutants of Hainan toxin-IV. Chin. J. Biotechnol., 2005, 21, 92-96.

[31] Fei, R.; Yang, Y.; Zhang, L.J.; Gao, J.C.; Zhou Y.Z. Advance research and application of spider venom. J. Jilin Uni. (Med. Ed.), 2004, 30, 994-996.

[32] Liu, R.Y.; Chen, J.Q.; Wang, Y.R.; Mao, H.F. Inhibitory effects of Huwen toxin-Ⅰ on the Fas apoptosis pathway of hippocampal neurons in rats with global cerebral ischemic reperfusion injury. Chin. J. Sports Med., 2007, 26, 704-708.

[33] Ren, W.H.; Zhang, S.Q.; Song, D.X.; Zhou, K.Y. Purification of the haemolytic peptide from the venom of Scolopendra subspinipes mutilans. Curr. Zool., 2007, 53, 519-523.

[34] Zhang, Y.H.; Si, Q.J.; Wang, X.G. Study on anti-atherosclerosis in rabbit from the centipede. Pharmacol. Clin. Chin. Mater. Med., 2005, 21, 26-27.

[35] Mao, X.P.; Chen, Z.J.; Mao, X.J.; Xiao, Q.C.; Dai, R. Study on the pharmacological of centipede. J. Yunnan College Trad. Chin. Med., 1999, 22, 1-3,7.

[36] Chen, S.P.; Han, Y.L.; Guo, Z.; Li, X.N. Study on the antithrombotic effect of fibrinolytic proteins from Scolopendra subspinipes mutilans. Chin. Pharmacol. Bull. 2007, 23, 1088-1092.

[37] Wang, L.N.; He, L.; Cheng, H.; Jian, W.; Wang, J.; Hu, J.P. Effects of centipede extraction on vWF and TPO expression in blood plasm after focal cerebral ischemia-reperfusion injury in rats. Chin. J. Exper. Trad. Med. Formulae, 2012, 18, 192-195.

[38] Malta, M.B.; Lira, M.S.; Soares, S.L.; Rocha, G.C.; Knysak, I.; Martins, R.; Guizze, S.P.; Santoro, M.L.; Barbaro, K.C. Toxic activities of Brazilian centipede venoms . Toxicon, 2008, 52, 255-263.

[39] Peng, Y.G.; Li, L.D.; Deng, Y.H. Effect of batryticated silkworm on experimental venous thrombosis and its mechanism. Chin. J. Throm. Hemost., 2001, 7, 104-105.

[40] Wang, J.H. The pharmacological research and clinical application of batryticated silkworms and muscardine pupae. Lishizhen Med. Mater. Med. Res., 2003, 14, 492-494.

[41] Hu, Y.Q.; Tang, N.; Liu, T.; Liu, Z.J.; Zhu, M.Z.; Hu, Y.Y.; Fan, L.L. Effect of Qingre Huayu presciption on expression of glial cell line-derived neurotrophic fator mRNA and protein in rats after cerebral ischemia/reperfusion. Chin. J. Integr. Med. Cardio-Cerebrovasc. Dis., 2011, 9, 1088.

[42] Hu, G.H.; Long, H.J.; Chen, B.Y.; Long .H.L. Protection of Qingre Huayu decoction on localized celebral ischemia reperfusion in rats. J. Trad. Chin. Med. Univ. of Hunan, 2006, 26, 7-9.

[43] Sun, R.; Wang, R.Q.; Yu, X.; Yin, J.W.; Qin, S.J. Modern research on Moschus about chemical substances and pharmacological activity. J. Pharmaceut. Res., 2005, 24, 296 -297.

[44] Lv, L.L.; Zhang, Z.P.; Huang, W.; Ren, H.Y.; Sun, R. The effects of muskone on blood brain barrier transferring function on MCAO model in rats. Pharmacol. Clin. Chin. Mater. Med., 2009, 25, 31-35.

[45] Li, F.J.; Xie, S.L. Protection of Xingnao Jing Injection on cerebral ischemia-reperfusion injury of rats. J. Chin. Med. Mater., 2011, 34, 1111-1113.

[46] Xia, X.H.; Liu, Y.M.; Zhao, G.F.; Peng, S.Q.; Xu, Q.Y.; Shen, Q. Effect of musk with borneol on brain water content and blood- brain barrier permeability in the rat model of cerebral focal ischemia with reperfusion. Chin. J. Exper. Trad. Med. Formulae, 2009, 15, 42-45.

[47] Zhou, W.T.; Hamulati, W.P.E.W.; Niwaer, W.M.E. The progress of experimental study on antithrombotic traditional Chinese medicine. J. Chin. Med. Mater., 2011, 53, 1468-1473.

[48] Jing, R. Mexican scientist have found that the bat's saliva can treat thrombus. Chin. J. Animal Husb. Veter. Med., 2003, 19, 58-59.

[49] Yan, X.B.; Wang, L.J.; Wang, Y. The effect of Apistoxin injection on 86 cases of rheumatic and rheumatoid arthiritis. Chinese Journal of Integrative Medicine, 1995, 6, 370.

[50] Zhao, Y. Eds. Annual Report of the Key Laboratory of Medical Insects and Spiders Resources for Development and Utilization at Yunnan Province. (2013, Obtober 18). 44pp. Personal communications.