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ORIGINAL ARTICLE

Experimental Study on the Protection of Agrimony Extracts from Different Extracting Methods against Cerebral Ischemia-Reperfusion Injury[△]

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Objective To study the protective effect of agrimony extracts from different extracting methods on cerebral ischemia-reperfusion injury in rats, in order to optimize the extraction scheme of agrimony.

Methods Male rats were randomly assigned into seven groups: 1. Sham-operated group, 2. Untreated MCAO group (MCAO), 3. Petroleum ether extract of *Agrimonia pilosa* treated MCAO group (PEA), 4. Ethyl acetate extract of *Agrimonia pilosa* treated MCAO group (EAEA), 5. Ethanol extract of *Agrimonia pilosa* treated MCAO group (EEA), 6. Water extract of *Agrimonia pilosa* treated MCAO group (WEA), 7. Nimodipine treated MCAO group (NP). Intragastrical drug administration (i.g) was performed at 0 and 6 hours after MCAO. Neurological function tests were performed after reperfusion for 24 hours, then the brain was removed for the evaluations of the cerebral infarction volume (percentage of total brain volume) by immunohistochemistry, histological changes (hematoxylin-eosin staining), Na⁺/K⁺-ATPase, Ca²⁺-ATPase (modified method of Svoboda and Mosinger), mRNA expression of Tumor suppressor gene (P53) and heat shock protein (HSP70) (quantitative real-time PCR).

Results The neurological function of MCAO group had significantly higher scores than the sham group ($P<0.01$). The WEA group showed a significantly lower neurological score than the MCAO group ($P<0.05$), indicating the protective effect of WEA on neurological deficits. The mean infarction volumes of WEA ($13.5\pm6.6\%$, $F=4.75$, $P<0.01$), EEA ($19.90\pm6.90\%$, $F=5.23$, $P<0.01$), PEA ($20.40\pm5.30\%$, $F=4.68$, $P<0.01$) and EAEA ($22.50\pm10.50\%$, $F=6.25$, $P<0.05$) group were all significantly smaller than that of MCAO group ($29.40\pm6.50\%$). HE staining demonstrated that, compared to the treated groups, the infarcted cerebral

tissue of MCAO group had more swelling neural cells, lighter stained nucleus, fewer and irregularly distributed neurons. The activity of Na^+/K^+ -ATPase and Ca^{2+} -ATPase reduced in the MCAO group (3.67 ± 0.48 U/mg, 1.28 ± 0.26 U/mg, respectively), and were significantly higher in WEA group (7.56 ± 0.85 U/mg, $F=12.65$, $P=0.010$; 3.59 ± 0.22 U/mg, $F=8.32$, $P=0.041$, respectively). The MCAO group showed significantly elevated P53 and HSP70 mRNA expressions compared to the sham group ($P<0.01$, $P<0.05$). P53 mRNA expressions in Agrimony extracts treated groups were significantly lower than that of the MCAO group (all $P<0.01$), with the WEA group showing the greatest difference from MCAO group. The HSP70 mRNA level of the treated groups were not significantly different from that of the MCAO group.

Conclusions Treatment using water extracts of agrimony can promote the best functional and metabolic recovery for rat model of cerebral ischemia-reperfusion injury, which maybe relate with the upregulation of energy metabolism in nerve cells after MCAO.

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CEREBRAL ischemia-reperfusion injury, a life-threatening disease, refers to ischemia-induced cerebral cell damage that is aggravated by restoring the blood supply.¹ Tissue damage after stroke involves multiple deleterious mechanisms, and existing therapeutic strategies, such as interventional procedures and surgery, have limitations.²⁻⁴ Novel therapeutic methods for ischemia stroke are needed to be explored.

Traditional Chinese Medicine (TCM) has the advantage of comprehensive treatment effects on multi-sites, multi-targets and overall regulation in ischemia-reperfusion injury. *Agrimonia eupatoria*, a well investigated traditional medicinal plant, has been largely used in the treatment of headache, abdominal pain, sore throat, bloody discharge, parasitic infections and eczema in China and other Asian countries. Studies have found that *Agrimonia pilosa* has functions of anti-oxidative, anti-inflammatory, improving lipid metabolism and regulating apoptosis.^{5,6} The active components of *Agrimonia pilosa* are mainly agrimony, agrimonolide, tannin, organic acids, saponin, etc.^{7,8} Because of the complex composition of agrimony grass, which contains phenol, ester, glycosides, flavonoids, organic acids, and other ingredients, different extraction methods usually get different effective ingredients.

Previous studies on agrimony are mainly in the following aspects: 1. Acetylcholinesterase inhibitory effects;⁹ 2. Free radical scavenging and DNA oxidative damage protecting activities;¹⁰ 3. Antioxidant activity and aldose reductase inhibitory potential;¹¹ 4. Glucosidase inhibitory activity of the flavonoid compound and the triterpenoid compound.¹² Although *Agrimonia pilosa* treatment, as mentioned above, can improve metabolism and prevent apoptosis after middle cerebral artery occlusion (MCAO), to the best of our knowledge, no publication has compared the therapeutic effects of the extracts of *Agrimonia pilosa* from different

extraction methods. Thus, in the present study, we used neurological function tests, 2,3,5-triphenyltetrazolium chloride (TTC) staining, histologic analysis and quantitative real-time PCR to investigate on a rat model of MCAO the therapeutic effects of extracts of *Agrimonia pilosa* from different extracting methods, aiming at optimizing the effective extracting methods and exploring the mechanism of *Agrimonia pilosa* in protecting rat from cerebral ischemic-reperfusion injury.

MATERIALS AND METHODS

Animals and experimental design

This study has been approved by the local Institutional Animal Care and Use Committee. Adult male SD rats (SCXK2014-0001) weighing 240–270 g were housed in a colony room under conditions of 12 h light/dark cycles and having free access to food and water. All rats were randomly divided into seven experimental groups (8 rats for each group): 1. Sham-operated group, 2. Receiving a transient middle cerebral artery occlusion (MCAO) surgery without treatment, 3. MCAO with treatment of petroleum ether extract of *Agrimonia pilosa* (PEA group), 4. MCAO with treatment of ethyl acetate extract of *Agrimonia pilosa* (EAEA group), 5. MCAO with treatment of ethanol extract of *Agrimonia pilosa* (EEA group), 6. MCAO with treatment of water extract of *Agrimonia pilosa* (WEA group), 7. MCAO with treatment of nimodipine (NP group). Intragastrical administrations were performed at 0 and 6 hours after MCAO at a dosage of 4g/kg for extracts of *Agrimonia pilosa* and 0.5mg/kg for nimodipine. Behavioral tests were performed after reperfusion for 24 hours. All rats were euthanized 24 hours after MCAO and brains were removed for histological and immunohistochemical examinations.

Plant material and extraction

The *Agrimonia pilosa* was acquired in Zhejiang Province, China (Batch number: 140922) and was identified according to the Flora of China. 100g dried *Agrimonia pilosa* was treated with heating reflux thrice, and then the product was mixed with 10 volumes of petroleum ether, ethyl acetate, 95% ethanol or water for 1 hour. The extracts were filtered through filter paper (Whatman, Maidstone, UK) and concentrated by a rotary evaporator (EYELA, Tokyo, Japan) under reduced pressure at 40°C until reaching a concentration of 0.4g/ml. The yield of each part was: ethyl acetate 5.53%, ethanol 7.40%, water 15.77%, ether 4.62%. Each extract was digested with 2.0% Tween-80 to prepare the extracts for a concentration of 0.4 g/ml, and was stored at 4°C till use.

Procedure of middle cerebral artery occlusion and reperfusion

The MCAO model was induced by the intradermal suture technique as previously described.¹³ Briefly, all rats were anesthetized with 1.5% pentobarbital sodium (50mg/kg, intraperitoneal injection). Make a midline skin incision in the neck, and expose the right common carotid artery (CCA), the external carotid artery (ECA), and the internal carotid artery (ICA). A 3-0 monofilament nylon suture was introduced to right common carotid artery and advanced into the internal carotid artery approximately 18-20mm intracranially from the common carotid artery bifurcation. The thread was left in place for 90 minutes to block the blood supply of the right middle cerebral artery. Then the suture was removed for reperfusion. The temporal muscle and skin were sutured with 4-0 nylon threads. Continuously monitored rectal temperature and maintained it within 36.5°C-37.5°C during the surgery.

Neurological function test

Animals of each group were subjected to neurological function test 24h after MCAO using the Zea Longa neurological grading system by a dedicated investigator who was blinded to the experimental groups. Briefly, neurologic findings were scored on a five-point scale:¹⁴ 0, no neurologic deficit; 1, mild focal neurologic deficit; 2, moderate focal neurologic deficits; 3, severe focal deficits; 4, unable to walk spontaneously with defective consciousness.

TTC staining and infarct volume measurement

Rats (5 per group) were euthanized by decapitation. The brains were sliced coronally into 2mm slices starting 1mm from the frontal pole. The tissue sections were immediately

incubated in 1.5% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma, St. Louis, USA) at 37°C for vital staining, and fixed in 4% paraformaldehyde at 4°C overnight before analysis. Brain slices were scanned using a flat-bed scanner. After taking pictures, the infarct area and total area of each brain slice were quantitatively analyzed using the Motic Digital Microscopic Graphic Analysis System (Motic Images Advanced 3.2). The volumes of the infarct tissue and the whole brain were then calculated. The percentages of the infarct volume in whole brain were compared among each group.¹⁵

Hematoxylin-eosinstaining(HE) staining and histological evaluation

The brain was fixed by transcardial perfusion with a buffered 4% paraformaldehyde solution before paraffin-embedded. After that, 4 μ m coronal serial sections were processed with a sliding microtome (HM-340 E, Microm, Walldorf, Germany) throughout the ischemic penumbra cortex. Regular hematoxylin-eosin staining was conducted, and the histopathological changes were observed under a light microscope.

Measurement of Na⁺/K⁺-ATPase and Ca²⁺-ATPase activities

The rats were sacrificed under deep anesthesia, and brains were immediately removed, dissected and homogenized in 50 mM phosphate buffers (pH 7.0) containing 0.1 mM duodenum edentate (EDTA) to yield 2% (w/v) homogenate. The homogenate was centrifuged at 2,500 rpm for 10 min at 0°C, and the supernatant was used to determine oxidative product contents and the antioxidant enzyme activities using a BCA protein assay kit (Beyotime Institute of Biotechnology, China).

Modified method of Svoboda and Mosinger¹⁶ was used to measure the activities of Na⁺/K⁺-ATPase and Ca²⁺-ATPase. The activities were determined by reaction in media A (test mixture) and media B (standard mixture). Media A contains 1.0 M NaCl, 0.2 M KCl, 0.1 M MgCl₂, 0.2 M Tris-HCl (pH 7.4) and 0.1 ml of brain homogenate supernatant in a total volume of 2.0 ml. Reaction mixture B contains 10 mM ouabain, 1.0 M NaCl, 0.1 M MgCl₂, 0.2 M Tris-HCl (pH 7.4) and 0.1 ml of brain homogenate supernatant in a total volume of 2.0 ml. Then 0.2 ml of 25.0 mM ATP was added to both A and B mixture at 37°C for 15min. After that, 1.0 ml of chilled 10% trichloroacetic acid (TCA) was added to the above mixtures to terminate the reaction. The mixtures were centrifuged at 2,500 rpm for 10 min, and 0.2 ml of supernatant was used for the estimation of inorganic phosphorous (Pi).

Quantitative real-time PCR analysis

Rats were sacrificed under deep anesthesia 24h after ischemia and reperfusion. Brains were immediately removed. Small amount of ischemic brain tissue was collected and placed in liquid nitrogen. Then the frozen tissue was ground into powder, and 50-80mg of the powder was used for total RNA extraction using the TRIzol reagent (BioBasic Inc., Canada).

We took 1 µg of total RNA to synthesize the first strand of cDNA using oligoT primer and MLV reverse transcriptase (Takara, Japan). Primers of P53, Heat shock protein 70(HSP70) and GAPDH were designed with Primer Premier 5.0 and synthesized by Shanghai Sangon Biotech (Table 1). A quantitative real-time PCR system (CFX384, Bio-Rad, USA) and SYBR Premix Ex Taq (Takara, Japan) were use for PCR reaction following the protocol: 95°C for 1 min, 40 cycles at 95°C for 15 sec, 63°C for 25 sec and 72°C for 60 sec. A melt curve analysis was performed to ensure specific amplification. For each target gene, the relative level of expression was normalized against the housekeeping gene GAPDH of the same sample.

Statistical analysis

One way ANOVA was adopted to compare the neurological function score, the infarction volume, the activity of Na⁺/K⁺-ATPase, Ca²⁺-ATPase, P53 and HSP70 mRNA expression among the groups. Data were presented as mean±SD, and difference was considered significant if *P*<0.05. Statistical analyses were performed using SPSS software (version 15.0, SPSS Inc.)

RESULTS

Effects on infarction volume

Rats in MCAO group were found to have significantly largest infarction volume (29.40±6.50%), and those in NP group had the smallest infarct volume (9.20±2.20%). Compared to the MCAO group, significantly less infarction volumes were observed in WEA, EEA, PEA and EAEA rats, which were 13.50±6.60% (*F*=4.75, *P*<0.01), 19.90 ± 6.90% (*F*=5.23, *P*<0.01), 20.40±5.30%, (*F*=4.68, *P*<0.01), and 22.50±10.50% (*F*=6.25, *P*<0.05), respectively. There was no infarcted tissue in the brains of sham group mice (Fig. 1, Fig. 2).

Table 1. Primer sequences of rat's genes: P53, Hsp70 and GAPDH

Primer	Exon/cDNA	Fragment size (bp)	Sequence (5'- 3')	
P53	P53	158	Forward	GGCTCCGACTATACCACTATCCACT
			Reverse	GGACAGGCACAAACACGAACCTCA
Hsp70	Hsp70	74	Forward	GCAGTGACCTTTCCTGTCCATTC
			Reverse	GTGCTGCTGTTGACACTGTTGCTAT
GAPDH	GAPDH	105	Forward	GAATCCCAGTAAGTGC GGTCATA
			Reverse	CGAGGGCCTCACTAAACCATC

P53, tumor suppressor gene 53; HSP70, hot shock protein 70; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Figure 1. Gross specimen observation on cerebral infarction of MCAO mice with and without treatment (TTC staining). A. Sham-operated group; B. MCAO mice without treatment; C. PEA, MCAO mice treated with petroleum ether extract of *Agrimonia pilosa*; D. EAEA, MCAO mice treated with ethyl acetate extract of *Agrimonia pilosa*; E. EEA, MCAO mice treated with ethanol extract of *Agrimonia pilosa*; F. WEA, MCAO mice treated with water extract of *Agrimonia pilosa*; G. NP, MCAO mice treated with nimodipine.

Figure 2. Comparison of cerebral infarction volume 24 hours after transient MCAO. * $P<0.01$, compared with Sham operated group; # $P<0.01$, compared with MCAO group; Δ $P<0.05$, compared with MCAO group.

Histopathological changes

HE staining showed that, in the sham group, nucleus and cytoplasm dyed clearly and were distributed evenly. In the MCAO group, volume shrink and swelling occurred in cells; the dye of nucleus was light; the distribution of cells was irregular; and the number of neurons decreased. In the treatment groups, the volume of swollen brain cells reduced; the number of neurons increased; and the nuclear staining was darker (Fig. 3).

Effect on neurologic functional recovery

The neurological function evaluation showed that the MCAO group had significantly higher scores compared to the sham group ($P<0.01$). Among the treated groups, only the WEA group had significantly lower neurological score than the MCAO group ($P<0.05$), indicating its protective effect on preventing MCAO mice from neurological deficits (Fig. 4).

Effect on $\text{Na}^+/\text{K}^+-\text{ATPase}$ and $\text{Ca}^{2+}-\text{ATPase}$ activities

Compared to the sham-operated group, substantial reductions in the activity of $\text{Na}^+/\text{K}^+-\text{ATPase}$ and $\text{Ca}^{2+}-\text{ATPase}$ were detected in the MCAO group. Among the treated groups, the activities of $\text{Na}^+/\text{K}^+-\text{ATPase}$ of WEA group (7.56 ± 0.85 U/mg, $F=12.65$, $P=0.010$) and NP group (7.59 ± 1.02 U/mg, $F=10.36$, $P=0.001$) were significantly higher than MCAO group. The activity of $\text{Ca}^{2+}-\text{ATPase}$ in WEA group (3.59 ± 0.22 U/mg, $F=8.32$, $P=0.041$), NP group (3.23 ± 1.03 U/mg, $F=8.82$, $P=0.031$) and EAEA group (3.86 ± 1.06 U/mg, $F=13.65$, $P=0.008$) were also higher than that of MCAO group (Table 2).

mRNA expressions of P53 and HSP70 in ischemic brain tissue

The MCAO group showed significantly elevated P53 mRNA expression compared to the sham-operated group ($P<0.01$). All groups treated with *Agrimonia pilosa* extractions and

the NP group had significantly lower P53 mRNA levels than the MCAO group ($P<0.05$) (Fig. 5); and that of the WEA group (39.49 ± 10.84) was different from that of the MCAO group more significantly than the other treated groups ($P<0.01$).

Figure 3. Histological observation on brain tissue of the experimental groups (HE staining $\times 100$).

A. Sham-operated group; B. MCAO group (untreated); C. PEA treated group; D. EAEA treated group; E. EEA treated group; F. WEA treated group; G. NP treated group.

Figure 4. Neurological function evaluation of the experimental groups.

Significant neurological function improvement was detected in the WEA group at 24 hours after transient MCAO; * $P<0.01$, compared with Sham group; # $P<0.05$, compared with MCAO group.

The HSP70 mRNA level of the MCAO, PEA, EEA and WEA groups were significantly higher than that of the sham group ($P<0.01$), and that of EAEA and NP group were also higher than that of the sham group ($P<0.05$). There was no statistic difference in HSP70 mRNA expression between MCAO group and any treated group (Fig. 6).

Table 2. Na^+/K^+ -ATPase and Ca^{2+} -ATPase activity of the experimental groups ($n=8$)

Group	Na^+/K^+ -ATPase (U/mg prot/hour)	Ca^{2+} -ATPase (U/mg prot/hour)
Sham	10.25 ± 1.28	4.45 ± 0.39
MCAO	$3.67 \pm 0.48^*$	$1.28 \pm 0.26^*$
PEA	4.42 ± 1.25	2.64 ± 0.44
EAEA	6.20 ± 1.09	$3.86 \pm 1.06^\Delta$
EEA	5.48 ± 0.93	1.21 ± 0.53
WEA	$7.56 \pm 0.85^\Delta$	$3.59 \pm 0.22^*$
NP	$7.59 \pm 1.02^\Delta$	$3.23 \pm 1.03^*$

* $P<0.01$ compared with the sham operated group; $^\Delta P<0.01$ compared with the MCAO group; $^*P<0.05$ compared with the MCAO group.

Figure 5. Comparison of P53 mRNA expression 24 hours after transient MCAO among experimental groups.

* $P<0.01$, compared with the sham operated group;
 $^\Delta P<0.01$, compared with the MCAO group.

Figure 6. Comparison of HSP70 mRNA expression 24 hours after transient MCAO among experimental groups.

* $P<0.01$, compared with the sham operated group;
 $^\Delta P<0.05$, compared with the sham operated group.

DISCUSSION

Since ischemic stroke-induced brain injury often causes irreversible brain damage, it is the worldwide leading cause of death, disability, and massive socioeconomic loss.¹⁶ Traditional Chinese medicines (TCMs) have successfully been used for centuries to treat stroke, and attract increasing attention from the industry and academia.¹⁷⁻²⁰

Stroke generally involves so many different deleterious processes and biochemical pathways that eventually lead to cellular injury and cell death following reperfusion. A large number of evidence from biochemical and pathological studies of animal models (transgenic and toxin) of neurodegeneration suggests that mitochondrial dysfunction is a common pathological mechanism after stroke. Mitochondrial dysfunction from oxidative stress, mitochondrial DNA deletion, pathological mutation, altered mitochondrial morphology, and interaction of pathogenic proteins with mitochondria leads to neuronal dysfunction and demise.²¹

The activity of Na^+/K^+ -ATPase and Ca^{2+} -ATPase represents the degree of neuronal injury. After cerebral ischemia-reperfusion injury, mitochondrial permeability increase, leading to more flow of Ca^{2+} in cerebral cell and increased conversion of xanthine dehydrogenase and xanthine oxidase, resulting in increase of free radicals, causing structural damage to chondrocytes,^{22,23} then aggravating chondrocyte respiratory dysfunction and energy metabolism failure. Finally, a severe cycle of oxygen free radicals occurs.^{24,25} Thus, the role of Na^+/K^+ -ATPase activity becomes more important in the secondary injury of neurons after ischemia.^{26,27} In addition, with the reduction or dysfunction of Cyt C, the mitochondrial respiratory chain becomes destroyed, and ATP synthesis decreases, eventually leading to cell death.²⁸ Therefore, cerebral cell become an important target of neuroprotection after cerebral ischemia-reperfusion injury.²⁹

In this study, the activity of Na^+/K^+ -ATPase and Ca^{2+} -ATPase decreased in the brain of rats 24 hours after ischemia-reperfusion, indicating the changes of oxygen free radicals and energy metabolism. In MCAO group, the activity of Na^+/K^+ -ATPase and Ca^{2+} -ATPase decreased significantly, demonstrating the important role of cellular energy metabolism in cerebral ischemia-reperfusion injury. On the other hand, the study found that among the treatment groups, WEA group maintained the highest activity of Na^+/K^+ -ATPase and Ca^{2+} -ATPase ($P<0.05$, respectively), which indicates that water extract of *Agrimonia pilosa* is the most effective in preventing from the decrease of Na^+/K^+ -ATPase and Ca^{2+} -ATPase in the cerebral ischemia-reperfusion injury, and therefore helps to improve energy

metabolism of chondriosomes in nerve cells after cerebral ischemia-reperfusion injury.

MacManus *et al*³⁰ for the first time found that there was an apoplectic component for selective neuronal death following global ischemia in rat brain in 1993. Recent studies have demonstrated that cerebral ischemia induced neuronal apoptosis by activating various signal proteins and mediating several signal pathways.^{31,32} Neuronal apoptosis after cerebral ischemia-reperfusion injury is regulated by a large number of apoptosis-related genes, which can be activated *via* related signal transduction pathways and induce apoptosis by a line of cascade reactions.

Study has shown that *Agrimonia pilosa* has the function of inducing apoptosis.³³ In the present study, we use apoptosis-related genes P53 and HSP70 as the key indicators to monitor the effects of different extracts of *Agrimonia pilosa* on regulating their expression in cerebral ischemia-reperfusion injury, and explore the neuroprotective function and mechanisms of *Agrimonia pilosa* in cerebral ischemic injury.

Previous studies have found a few reactive genes expressed after ischemia, including P53, which is related to DNA injury and repair.³⁴ The expression of P53 is upregulated in a variety of cerebral ischemic animal models, and it acts as an anti-apoptosis factor.³⁵ In addition, P53 promotes apoptosis through independent transcription pathways and triggers the apoptosis process by activating Bax mitochondrial translocation.³⁶ Mihara *et al*³⁷ showed that P53 protein could directly induce permeabilization of the outer mitochondrial membrane by forming complexes with the protective Bcl-xL and Bcl2 proteins, resulting in cytochrome c release.

HSP70 is a kind of highly conserved protein. Under normal circumstances, HSP70 exists in the cytoplasm, and its expression level is low. HSP70 is activated by a signal transduction pathway after cerebral ischemia-reperfusion injury, and is closely related to ischemic brain damage. There are two mechanisms about HSP70 for brain cell protection: (1) HSP70 facilitates the repair of denatured protein, and promotes the fold and stretch of newly formed polypeptide. (2) HSP70 can bind hydrophobic fraction of unfolded polypeptide and help the fold progress.³⁸ As soon as cerebral ischemic and hypoxia occur, oxidative phosphorylation is inhibited in chondriosomes, which decreases the production of ATP. HSP70 rapidly moves into the nucleus, surrounds the nucleolus, and the synthesis rate of HSP70 increases significantly.

Our data showed that expression of P53 and HSP70 significantly increased after cerebral injury in the rat model. Moreover, the expression of P53 was down-regulated in the

four groups treated with *Agrimonia pilosa* extracts. However, no difference in HSP70 mRNA expression was detected between MCAO group and the extracts-treated groups. These results suggested that *Agrimonia pilosa* might protect cerebral cells from ischemia-reperfusion injury through regulation of cell apoptosis. Whether HSP70 was involved in the protective mechanism need to be determined in future, and further experiments with large sample are needed to better understand the role of apoptosis in the protective mechanism of *Agrimonia pilosa*.

Based on our study, pharmacotherapy at the early stage of ischemia-reperfusion is helpful in reducing acute ischemic injuries. The results were consistent with the treatment outcome in clinic. Besides, the mechanism of WEA in treating cerebral ischemia-reperfusion injury is related to improving the function and energy metabolism of chondriosome, which is beneficial to the recovery of Na⁺/K⁺-ATPase and Ca²⁺-ATPase activity. On the other hand, inhibiting the expression of apoptosis gene may also be promising for the treatment of cerebral ischemia, which may help to decrease infarction volume and benefit neurologic function recovery.

Since agrimony contains complex chemical constituents of agrimonin, flavonoids, tannins, organic acids and saponins. The water extract contains more agrimonin than other extracts, which contain more flavonoids. Therefore, according to the results of this study, we initially suggested that agrimonin might have effects of anti-apoptosis and improved the metabolic activity of cells. Pharmacodynamics study employing high performance of liquid chromatography to analyze extracts and isolate the effective compound of WEA will be conducted in future.

In conclusion, this study demonstrated a functional and metabolic recovery after WEA treatment in the rat model of cerebral ischemia. The neuro-protective effect of WEA on cerebral ischemic-reperfusion injury might be relevant with its antioxidant potential and upregulation of energy metabolism in neural cells after MCAO.

Conflict of interests statement

All authors declared no conflict of interests to disclose.

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