Saudi Journal of Biological Sciences (2017) **24**, 729–736

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Intervention action of total flavonoids from root of *Ilex pubescens* in cerebral ischemic tolerance with blood stasis

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Received 7 December 2016; revised 23 December 2016; accepted 6 January 2017
Available online 26 January 2017

KEYWORDS

Total flavonoids from MDQ;
Cerebral ischemic tolerance with blood stasis;
Expression of Bcl-2 protein;
Expression of Bax protein

Abstract The aim of this study was to explore the action characters of total flavonoids from MDQ on cerebral ischemic tolerance with blood stasis. Fully understanding the mechanism of action of total flavonoids from MDQ is helpful for the development of new drugs and the utilization of resources. Male Wistar rat model of blood stasis was established by injecting dexamethasone into the intramuscular side of the thigh. Then they were given related drugs via an intragastric administration for a successive 10 days. After 7 days, the following occurred: firstly, the method of blocking the bilateral common carotid artery (CCA) was used for 10 min, followed by a restoration of perfusion. After 72 h, we performed a temporary occlusion of the rat's middle cerebral artery for 2 h with an intraluminal thread method. This was followed by reperfusion for 24 h, respectively, to establish the rat model of cerebral ischemic tolerance with blood stasis. Viscosity of the whole blood was measured after the last administration was given blood. Brain was removed, and then the activity of ATP enzyme and T-SOD was determined. To observe the pathological changes of the hippocampus area by HE staining, and the expression of Bcl-2 and Bax were observed by immunohistochemical method. The rat model of cerebral ischemic tolerance with blood stasis was copied successfully. The whole blood viscosity, the activity of NOS, the content of Gluin in the ischemic brain in the IPC model group and the ischemia–reperfusion group were increased significantly. The activity of ATPase was decreased significantly. Compared with the ischemia–reperfusion model group, the activity of ATPase and the whole blood viscosity in the ischemic preconditioning (IPC) group were increased significantly. The activity of NOS and the content of Gluin were decreased significantly. The degree of pathological injury of the brain tissue was also relieved significantly. Total flavonoids of MDQ were used, improving blood circulation, improving

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2017.01.049

energy metabolism, activating endogenous anti-oxidative capability, enhancing the antiapoptotic effect, and relieving the injury of the nerve cell. Hence, the use of MDQ flavonoids improves the tolerance ability of cerebral ischemia.

1. Introduction

In China, the dried root of *Ilex pubescens* Hook. et Arn. (MDQ) aqueous extract can be used for parenteral administration for the treatment of cardiovascular diseases. At this point most research is geared toward the exploration of the pharmacological study and clinical application of the MDQ extract. There is only a small part of the total flavonoids of research. The mechanism research and application research have many contents that remain to be studied further.

MaoDongqing (MDQ) is the dried root of *Ilex pubescens* Hook. et Arn. It belongs to the family of Aquifoliaceae. The effect of MDQ is promoting blood circulation, relieving pain, and detoxification (Hao and Yang, 2010). It is a Chinese herbal medicine commonly used in Southern China. Its root has been well-known for its medicinal use in treating cardio cerebral, vascular and arterial thrombosis diseases (Wang, 2006; He and Qiu, 2010) such as stroke, coronary arterial thrombosis, thromboangiitis obliterans, and thrombophlebitis (Zheng et al., 2006). In addition, it has often been used for alleviating upper respiratory infections and other inflammatory diseases. Pharmacological studies showed that extracts of MDQ could significantly enlarge blood vessels, improve blood microcirculation, lower blood pressure, inhibit platelet aggregation (Jiang et al., 2005), prevent thrombosis, and reduce cardiac ischemia .It could also protect brain tissue and has anti-inflammatory properties. Previous studies showed that the total flavonoids from MDQ could improve the cerebral homogenate ATP enzyme activity, decrease the content of LD and reduce the MDA content. It could also reduce cerebral injury caused by ischemia. Furthermore, it had a good effect on the cerebral ischemia of the rat model (Cheng et al., 2012a,b; Miao, 2009). Preliminary experiments have found a more mature extraction process that can be used to purify the total number of flavonoids (Xu et al., 2011). This experiment observed the effect of total flavonoids from MDQ on hemorheology, brain tissue energy metabolism, SOD activity and apoptosis, as well as the anti-apoptotic gene in the rat models of cerebral ischemic tolerance with blood stasis.

2. Material and methods

2.1. Drug agents

Total flavonoids from MDQ (52% of content provided by analytical chemistry laboratory, Henan University of Chinese Medicine, batch number: 20101101); Extract of Ginkgo Biloba Leaves Tablets (Jin Naduo) (DR Willmar Schwabe production, batch number: 0601209); Dexamethasone Sodium Phosphate Injection (DX) (Lianshui Jiangsu Pharmaceutical Co., Ltd. production, batch number: 0912253); Sodium Chloride Injection (Zhengzhou Yonghe Pharmaceutical Co. Ltd., batch number: 10062121); Coomassie blue protein determination kit (batch number:20101214); ATP Adenosine-triphosphate detection reagent box (batch number:20101215); SOD (superoxide dismutase) detection reagent box (batch number: 20101213), both provided by Nanjing built biological engineering institute.

2.2. Instrument

LBY-N6A type rotary viscometer (Beijing precil group); 75-2 Spectrophotometer (Shanghai Third Analytical Instrument Factory).

2.3. Animals

Clean grade male Wistar rats, weight 280 g ~ 300 g, provided by Hebei experimental animal center, certificate number 1010133.

2.4. Methods

A total of 112 male, Wistar rats were randomly divided into 8 groups. They were given large, medium and small doses of total flavonoids from MDQ (0.2 g/kg, 0.1 g/kg, 0.05 g/kg; 10 mg/ml, 5 mg/ml, 2.5 mg/ml). The positive control drug, Ginaton, was given to one group (0.02 g/kg, 1 mg/ml). There was also a cerebral ischemia reperfusion injury group, a cerebral ischemic tolerance group, a blood stasis sham operation group, and a non-blood stasis sham operation group. The rat model of blood stasis was established by an intramuscular injection of dexamethasone (0.2 $mg{\cdot}kg^{-1}\ d^{-1}$) in the side of the thigh. Then they were given related drugs via an intragastric administration for successive 10 days. The Cerebral ischemia reperfusion injury group, cerebral ischemic tolerance group, blood stasis sham operation group and non blood stasis group were treated with the same volume of 0.1% sodium carboxymethyl cellulose. The volume of perfusion was 2 ml/100 g and the positive drug group was prepared with 0.1% (sodium carboxymethyl cellulose) CMC solution.

The non-blood stasis sham operation group was intraperitoneally (ip) injected with an equal volume of normal saline. The rest of the 7 groups were injected with dexamethasone 0.2 mg/kg (1 mL/kg) on the hind leg muscles and given related drugs once per day using the method of intragastric administration. After 7 days the following occurred. Firstly, we used the method of blocking the bilateral common carotid artery (CCA) for 10 min (Fang et al., 2010, 2009), and then restored perfusion. After 72 h, fasting occurred for 12 h. In the last hour of fasting we used the method of blocking the bilateral CCA. Then we used a thread to occlude the left middle cerebral artery for 2 h, followed by reperfusion for 24 h, respectively, to establish the rat model of cerebral ischemic tolerance with blood stasis.

After 24 h of a second operation, the blood was taken from the eyeball and the heparin was used to measure the rheology of the whole blood. The rats were sacrificed and then decapitated rapidly, stripping the whole brain on an ice plate. In the coronary position, we took the tissue blocks depending on the chiasma opticum, with ends measuring 3 mm–4 mm and a fixed solution of 10% formaldehyde. Then the morphological changes of the brain were observed with a section of Hematoxylin and eosin (HE) staining. The expression of Bcl-2 and Bax in the brain tissue was observed by immunohistochemical staining.

The other half of the brain was used to prepare brain homogenate, which was used to determine the activity of ATP, T-SOD according to the kit manual.

For data analysis we used SPSS 17.0 for windows for statistical treatment. The measurement of data is represented by mean ± variance (s). For group comparison we used analysis of variance; for ranked data used a Ridit test.

3. Results

3.1. The hemorheology in rats of each group

Compared to the cerebral ischemia reperfusion injury group, the cerebral ischemic tolerance group's mortality rates significantly dropped. The mortality rates also dropped significantly in the Ginaton group, the group with large doses of flavonoids and the group with medium doses of flavonoids. Compared with non-blood stasis sham operation group, the blood rheology of rats in the blood stasis sham operation group was increased significantly ($P < 0.01$). The results showed that the rat model of blood stasis induced by dexamethasone was successful. Compared with the blood stasis sham operation group, the level of whole blood with low shear viscosity rose significantly in the cerebral ischemic tolerance group ($P < 0.01$), However, there was no significant change in the whole blood rheology of the cerebral ischemia reperfusion injury group ($P > 0.05$). Compared with the cerebral ischemia reperfusion injury group, the levels of whole blood midst-shear viscosity rose significantly in the cerebral ischemic tolerance group ($P < 0.01$). These results show that the stimulation of the operation can cause the blood stasis degree to further increase. Compared with the cerebral ischemic tolerance group, Ginaton and the large doses of the flavonoids could reduce the viscosity of whole blood significantly ($p < 0.01$). The medium doses of flavonoids could also reduce the whole blood, low-shear viscosity significantly ($p < 0.01$), as well as clearly reduce the high-shear and midst-shear viscosity ($p < 0.05$). The small doses of flavonoids could reduce the whole blood low-shear and midst-shear viscosity significantly ($p < 0.01$). These results demonstrate that the total flavonoids from MDQ could improve the blood viscosity of the rat models of cerebral ischemic tolerance with blood stasis, as shown in Table 1.

3.2. The brain energy metabolism and antioxidant capacity in rats of each group

Compared with the non-blood stasis sham operation group, there was no significant change in ATP enzyme and T-SOD activity in the brain tissue of the blood stasis sham operation group ($P > 0.05$). Compared with the blood stasis sham operation group, the ATP enzyme and T-SOD activity of rats decrease significantly in the cerebral ischemia reperfusion injury group ($P < 0.01$). These results show that the cerebral ischemia and reperfusion can damage the brain cells, decrease the respiratory function of mitochondria and decrease the energy metabolism of the brain. Compared with cerebral ischemia reperfusion injury group, the activity of T-SOD, Na^+-K^+-ATP and Ca^{2+}-ATP increased clearly in the brains of rats from the cerebral ischemic tolerance group ($P < 0.05$). These results show that the cerebral ischemic precondition can improve the energy supply of cells and enhance the oxidative phosphorylation of mitochondria. This can play a role in the protection of brain cells. Compared with the cerebral ischemic tolerance group, the Ginaton can significantly increase the activity of ATP in brain tissue of rats ($P < 0.01$). Large doses of total flavonoids from MDQ can also significantly increase the activity of T-SOD, Na^+-K^+-ATPase and Mg^{2+}-ATPase in brain tissue of rats ($P < 0.01$) and clearly increase the brain tissue Ca^{2+}-ATPase activity ($P < 0.05$). Medium doses of total flavonoids from MDQ can also significantly increase the activity of T-SOD and Na^+-K^+-ATPase in the brain tissue of rats ($P < 0.01$), as well as clearly increase the brain tissue Mg^{2+}-ATPase activity ($P < 0.05$). Small doses of total flavonoids from MDQ can also significantly increase the activity of Na^+-K^+-ATPase in the brain tissue of rats ($P < 0.01$). The results demonstrated that the ATP enzyme and T-SOD activity in the rat model of blood stasis and cerebral ischemia tolerance were improved by the total flavonoids of MDQ. Thus, total flavonoids of MDQ plays a role in cell protection. The results are shown in Table 2.

3.3. Expression of Bax and Bcl-2 in brain tissue of rats in each group

The immunohistochemical results were negative in the Bcl-2 expression in the brain tissue of rats in each group, as well as in the Bcl-2 in the brain nerve cell membrane and cytoplasm of the non-blood stasis sham operation group (Fig. 1) and blood stasis sham operation group (Fig. 2). In the cerebral ischemic reperfusion injury group (Fig. 3) and cerebral ischemic tolerance group (Fig. 4), the Bcl-2 staining of brain cells were negative in most of the cell membrane and cytoplasm, a few were weakly positive or positive. In the Ginaton group (Fig. 5) and the large dose of total flavonoids from MDQ group (Fig. 6), the expression of Bcl-2 was positive or weakly positive in most of the brain cell membrane and cytoplasm, a few were strongly positive. The expression of Bcl-2 was positive or weakly positive in the majority of cell membrane and cytoplasm of the middle dose of total flavonoids from MDQ (Fig. 7). The Bcl-2 expression of the small dose was weakly positive (Fig. 8) (Table 3).

The immunohistochemical results were negative in the Bax expression in the brain tissue of rats in each group. The Bax in the brain nerve cell membrane and cytoplasm of non-blood stasis sham operation group (Fig. 9) and blood stasis sham operation group (Fig. 10) rats were also negative. The Bax staining of the brain nerve cell membrane and the cytoplasm of the cells in the Cerebral ischemia reperfusion injury group (Fig. 11) was brown and was strongly positive. In the cerebral ischemic tolerance group (Fig. 12), the Bax staining of brain

Table 1 The hemorheology in rats of each group (smean ± se).

Group	Dose g/kg	*n*	Mortality %	Hemorheology in the whole blood/mPa·s		
				Low-shear (1/10)	Midst-shear (1/60)	High-shear (1/150)
Non-blood stasis sham operation	–	14	12.5	19.875 ± 3.668****	10.179 ± 1.681****	7.709 ± 1.777****
Blood stasis sham operation	–	14	12.5	26.009 ± 1.656$^{\star\#\#}$	12.601 ± 0.821$^{\star\star\#\#}$	9.92 ± 1.206$^{\#\#}$
Cerebral ischemia reperfusion injury	–	8	50.0	27.659 ± 1.992	12.35 ± 0.963**	9.558 ± 1.019
Cerebral ischemic tolerance	–	9	43.8	28.767 ± 1.056	14.346 ± 1.091**	9.873 ± 1.245
Ginaton	0.02	10	37.5	22.277 ± 3.339****	11.604 ± 1.228**	7.496 ± .0.882****
Large dose of total flavonoids from MDQ	0.2	11	31.3	21.835 ± 3.153****	12.158 ± 1.003**	7.059 ± 0.485****
Medium dose of total flavonoids from MDQ	0.1	9	43.8	24.77 ± 2.535***	12.925 ± 1.075*	8.597 ± 0.567*
Small dose of total flavonoids from MDQ	0.05	8	50.0	25.672 ± 3.497*	11.922 ± 1.487*	9.111 ± 1.654

Note, compared with cerebral ischemia reperfusion injury group $^{*}P < 0.05$ $^{**}P < 0.01$, compared with cerebral ischemic tolerance group $^{\star}P < 0.05$ $^{\star\star}P < 0.01$, compared with non-blood stasis sham operation group $^{\#}P < 0.05$ $^{\#\#}P < 0.01$.

Table 2 The brain energy metabolism and antioxidant capacity in rats of each group (smean ± se).

Group	Dose (g/kg)	*n*	Na^{+}-K^{+}-ATP (μmolPi/gHb/hour)	Mg^{2+}-ATP (μmolPi/gHb/hour)	Ca^{2+}-ATP (μmolPi/gHb/hour)	T-SOD (U/mgprot)
Non-blood stasis sham operation	–	14	7.347 ± 1.243	7.848 ± 1.175	5.304 ± 1.186	126.996 ± 27.475
Blood stasis sham operation	–	14	7.122 ± 1.354****	7.662 ± 1.92****	5.265 ± 1.536****	118.232 ± 27.879****
Cerebral ischemia reperfusion injury	–	8	3.657 ± 0.765*	4.388 ± 0.836	2.774 ± 0.476*	65.349 ± 17.659*
Cerebral ischemic tolerance	–	9	4.800 ± 0.91*	5.532 ± 1.111	3.809 ± 0.54*	88.476 ± 18.168*
Ginaton	0.02	10	6.979 ± 0.767****	7.492 ± 0.804****	5.09 ± 0.535****	121.384 ± 19.939****
Large dose of total flavonoids from MDQ	0.2	10	6.831 ± 1.685****	7.286 ± 2.136****	4.874 ± 0.769***	116.805 ± 29.389****
Medium dose of total flavonoids from MDQ	0.1	10	6.396 ± 1.665****	6.837 ± 0.827***	4.660 ± 1.006**	109.284 ± 18.807***
Small dose of total flavonoids from MDQ	0.05	9	6.262 ± 0.588***	6.699 ± 1.22**	4.512 ± 0.943**	103.155 ± 11.722**

Note, compared with cerebral ischemia reperfusion injury group $^{*}P < 0.05$ $^{**}P < 0.01$, compared with cerebral ischemic tolerance group $^{\star}P < 0.05$ $^{\star\star}P < 0.01$.

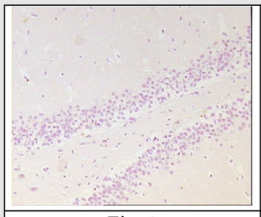
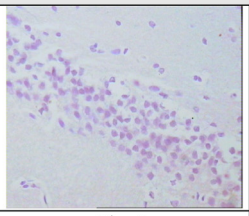
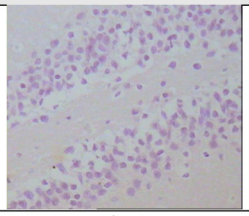
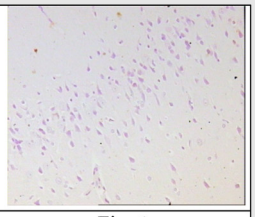
cells was positive in most of the cell membrane and cytoplasm, a few were weakly positive. In the Ginaton group (Fig. 12), the expression of Bcl-2 was weakly positive. In the Large dose of total flavonoids from MDQ group (Fig. 14), the expression of Bax was negative. In the Medium (Fig. 15) and Small (Fig. 16) dose of total flavonoids from MDQ, the Bax Expression of brain cells was negative in most of the cell membrane and cytoplasm. A few were weakly positive or positive. The Results are shown in Table 4.

The results of the Ridit test did not show any expression of Bcl-2 and Bax in the non-blood stasis sham operation group. Compared with the blood stasis sham operation group and the blood stasis sham operation group, the expression of Bax was significantly increased in the cerebral ischemia reperfusion injury group and cerebral ischemic tolerance group ($P < 0.01$). Compared with cerebral ischemic tolerance group, the expression of Bcl-2 was significantly increased in the large and medium dose of total flavonoids from MDQ ($P < 0.01$). The expression of Bax was significantly decreased in the large dose of total flavonoid from MDQ ($P < 0.01$), while the expression of Bax clearly decreased in the medium and small dose total of flavonoids from MDQ ($P < 0.05$). Compared with the cerebral ischemia reperfusion injury group, the expression of Bcl-2 was significantly increased in the cerebral ischemia reperfusion injury group and cerebral ischemic tolerance group ($P < 0.01$). The expression of Bax was significantly increased in each dose of total flavonoids from MDQ and Ginaton group ($P < 0.01$). The Results are shown in Table 5.

3.4. Changes of pathological in brain tissue of rats in each group

In the non-blood stasis sham operation group (Fig. 17), the brain nerve cells in the hippocampus CA1 area were basically normal. The brain nerve cells in the hippocampus CA1 area showed atrophy with eosinophilic degeneration and varying degrees of vacuolar degeneration. The brain nerve cells in the hippocampus CA1 area of the blood stasis sham operation group (Fig. 18) showed an obvious eosinophilic change. In the cerebral ischemia reperfusion injury group(Fig. 19) and cerebral ischemic tolerance group (Fig. 20), the brain neurons of the hippocampus CA1 area were showing signs of obvious degeneration and a small number of cell eosinophilic changes. The brain nerve cells in hippocampus CA1 area were basically normal in the large dose of total flavonoids from MDQ

Table 3 Expression of Bcl-2 in brain tissue of rats in each group IHC × 400.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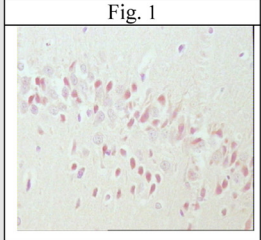
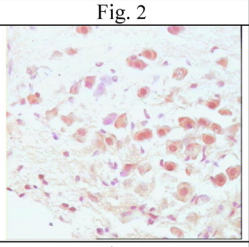
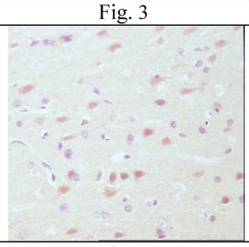
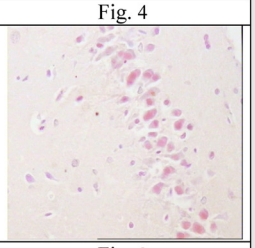
			

Fig. 5	Fig. 6	Fig. 7	Fig. 8

Table 4 Expression of Bax in brain tissue of rats in each group IHC × 400.

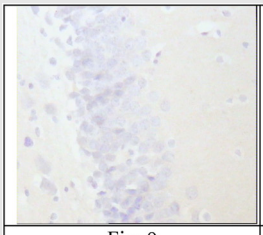
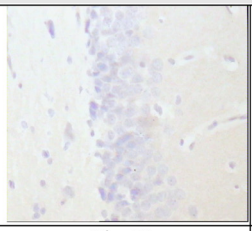
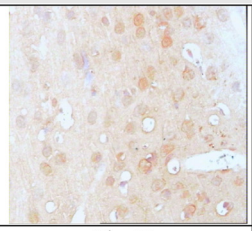
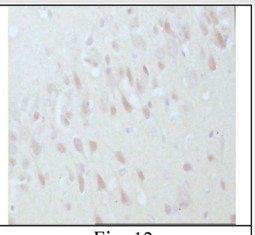
			

Fig. 9	Fig. 10	Fig. 11	Fig. 12

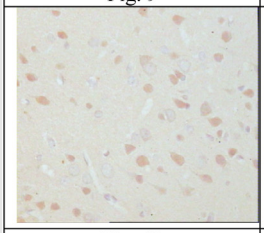
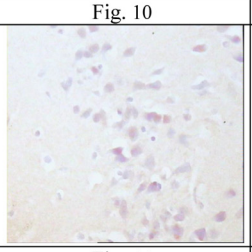
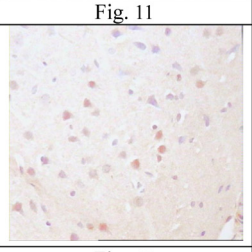
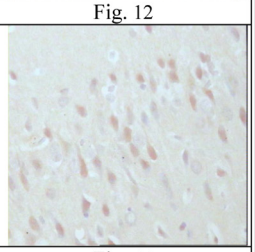

			

Fig. 13	Fig. 14	Fig. 15	Fig. 16

(Fig. 22) and Ginaton group (Fig. 21). In the Medium dose of total flavonoids from MDQ (Fig. 23), a small number of brain nerve cells showed a shrinking phenomenon. The individual cells showed an eosinophilic change. Most of the brain nerve cells showed significant atrophy in the small dose of total flavonoids from MDQ (Fig. 24) Table 6.

The results of the Ridit test show that compared with the blood stasis sham operation group, Cerebral ischemia reperfusion injury group and cerebral ischemic tolerance group had important statistical significance ($P < 0.01$) and demonstrate a successful model. Compared with the cerebral ischemic tolerance group, the pathological damage of brain tissue can be significantly reduced by Large dose of total flavonoids from MDQ ($P < 0.01$). Medium dose can clearly reduce the pathological damage of brain tissue ($P < 0.05$). The Results are shown in Table 7.

4. Discussion

Ischemic cerebrovascular disease has the characteristics of high incidence rates, high disability rates, high mortality rates and high recurrence rates. It is a common disease which is harmful to human life and health (Hartley et al., 2016; Sun et al., 2014). Cerebral ischemic tolerance (BIT) is a phenomenon that is induced by transient ischemic or mild hypoxia that stimulates or mobilizes the body's inherent ability to protect the body against subsequent, severe ischemia and hypoxia. Through the improvement of cerebral ischemic tolerance, the treatment time window of ischemic cerebrovascular disease is prolonged and the clinical sequelae of ischemic strokes are reduced. This is a new method for clinical prevention and treatment of ischemic cerebrovascular disease.

Table 5 Expression of Bax and Bcl-2 in brain tissue of rats in each group.

Group	Dose (g/kg)	*n*	–		+		++		+++	
			Bcl-2	Bax	Bcl-2	Bax	Bcl-2	Bax	Bcl-2	Bax
Non-blood stasis sham operation	–	14	14	14	0	0	0	0	0	0
Blood stasis sham operation	–	14	13	14	1	0	0	0	0	0
Cerebral ischemia reperfusion injury	–	8	7	0	1	0	0	0	0	8
Cerebral ischemic tolerance	–	9	6	2	2	2	1	5	0	0
Ginaton	0.02	10	1	5	3	5	6	0	0	0
Large dose of total flavonoids from MDQ	0.2	10	0	10	4	0	5	0	1	0
Medium dose of total flavonoids from MDQ	0.1	10	2	6	4	1	4	3	0	0
Small dose of total flavonoids from MDQ	0.05	9	6	5	3	2	0	2	0	0

Note, "–" Brain nerve cell membrane, cytoplasmic Bcl-2, Bax negative,"+" Brain nerve cell membrane, cytoplasmic Bcl-2, Bax weakly positive, "++" Brain nerve cell membrane, cytoplasmic Bcl-2, Bax positive,"+++" Brain nerve cell membrane, cytoplasmic Bcl-2, Bax strongly positive.

Table 6 Changes of pathological in brain tissue of rats in each group HE × 400.

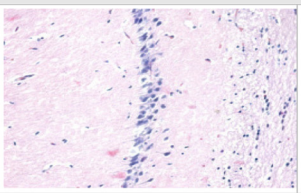
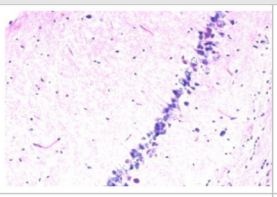


Fig. 17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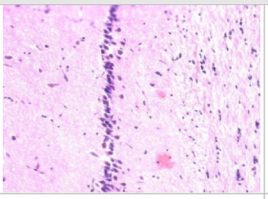
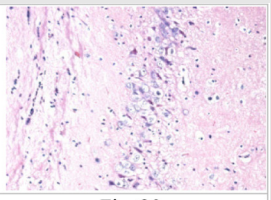


Fig. 19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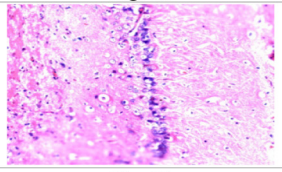
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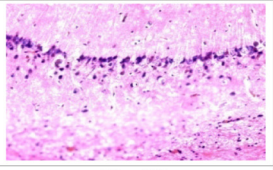
Fig. 22

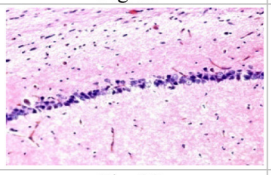
Fig. 23

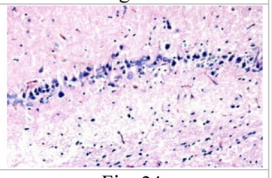
Fig. 24

Table 7 Changes of pathological in brain tissue of rats in each group.

Group	Dose (g/kg)	n	–	+	++	+++
Non-blood stasis sham operation	–	14	14	0	0	0
Blood stasis sham operation	–	14	14	0	0	0
Cerebral ischemia reperfusion injury	–	8	0	0	0	8
Cerebral ischemic tolerance	–	9	0	0	9	0
Ginaton	0.02	10	0	2	8	0
Large dose of total flavonoids from MDQ	0.2	10	8	2	0	0
Medium dose of total flavonoids from MDQ	0.1	10	6	2	2	0
Small dose of total flavonoids from MDQ	0.05	9	4	2	3	0

Note, "–" Brain nerve cells are normal, the cell cytoplasm is rich, the nucleus and the nucleus are clearly visible. "+" 20% Brain nerve cells showed a shrinking phenomenon, the individual cells are eosinophilic change. "++" 50% Brain nerve cells showed atrophy, with eosinophilic degeneration, individual cell showed vacuolar degeneration. "+++" 80% Brain nerve cells showed atrophy, with eosinophilic degeneration and vacuolar degeneration.

TCM believes that cerebral ischemia belongs to the category of an ischemic stroke of traditional Chinese medicine and that "stasis" is most closely related. "Promoting blood circulation to remove blood stasis" is a basic way to improve the cerebral ischemic tolerance. Promoting blood circulation and removing blood stasis of the disease has better clinical effects (Miao et al., 2009; Liu and Liu, 2010). It provides a basis for the intervention of traditional Chinese medicine on cerebral ischemia. With the development of the theories of Chinese Medicine, Cheng et al. (2012a,b) proposed the use of heat-clearing, detoxifying and promoting blood circulation to remove the blood stasis treatment of cerebral ischemia. This method not only combines the new theory of "removing toxin and unclog the meridian" in the treatment of cerebral ischemia, but also breaks the current situation of "promoting blood circulation to remove blood stasis" for the treatment of the

disease. This opens the way for the treatment of cerebral ischemic diseases. MDQ has the function of heat-clearing, detoxifying, Blood Activating and Meridian Dredging. Research shows that MDQ flavonoids could increase the activity of ATP in the brain tissue of cerebral ischemia model, decrease the content of lactic acid, improve the pathological changes of brain tissue, induce BIT and protect the brain tissue (Zhang et al., 2012) Other studies have found that extraction from MDQ can promote the expression of GAP 43 in brain tissue of ischemic, promote the repair and regeneration of neurons to protect nerve cells in the brain, reduce brain edema, inhibit lipid peroxidation, protect the brain and improve the BIT. (Sheng et al., 2009).

At present, there are many experimental studies on ischemic strokes. However, most of the cerebral ischemia models are using normal animals (Krzyzanowska et al., 2016; Wang et al., 2016). Although it can basically reflect the characteristics of cerebral ischemia disease, it failed to reflect the importance of "blood stasis." As a result, there is a certain gap between the clinical. According to our previous research results (Miao et al., 2007), the model used in this research is established on the basis of blood stasis, suitable degree of ischemia, a simply surgical method, a model with a high success rate, low death rate, and fits well with the clinical features. It is more suitable for the prevention and treatment of ischemic cerebrovascular disease in traditional Chinese medicine. BIT is a complex, biological process involving the regulation and control of a transmitter, receptor, channel, gene expression and protein synthesis. When cerebral ischemia occurs, blood viscosity increases, the velocity of blood flow slows down, blood oxygen content decreases, coupled with vasoconstriction and other factors. This can lead to reduced blood flow to the brain, lack of energy supply and tissue destruction of brain cells. ATP enzymes play an important physiological role in maintaining cell physiological activity, body temperature and normal metabolism. When cerebral ischemia occurs, ATP depletion, Ca^{2+} overload and membrane phospholipid degradation can cause the ATP enzyme activity to reduce. Widner reported that the decrease of intracellular magnesium ion is the inducing factor of irreversible cell damage. Mg^{2+}/Ca^{2+} ratio is an important index to reflect the irreversible damage. Therefore, the content of ATP can reflect the energy metabolism of brain cells in the brain after cerebral ischemia occurs.

It has been found that mainly B cell lymphoma proteins 2 (Bcl-2), Bcl-xl, Mcl-1 and so on, belonged to antiapoptosis genes, apoptosis genes, and mainly include Bax, Bak and so on. There is evidence that Bcl-2 can be used as an endogenous protective substance. The high expression of Bcl-2 can inhibit the cell death caused by many kinds of apoptosis factors and prolong the life span of the cells (Solaroglu et al., 2006). A study found that rat cerebral ischemia for 20 min can induce cerebral ischemic tolerance in rats. The expression of apoptosis gene Bcl-2 in neuronal cells also increased (Zhao et al., 2004). Bax is considered to be the most important apoptosis inducing gene (Eberspacher et al., 2003), which can prevent Bcl-2 from inhibiting apoptosis and promoting cell death and cell apoptosis. Through the study of the whole cerebral ischemia model in rats, Bax was found to be the cause of delayed neuronal death and the effect of Bcl-2 was just the opposite. Bax and Bcl-2 can form two polymers, which is directly related to the level of apoptosis regulation and the severity of apoptosis: Bcl-2 increased and inhibited apoptosis, while Bax increased and promoted apoptosis (Amemiya et al., 2005).

5. Conclusions

The results showed that ischemic preconditioning can induce cerebral ischemic tolerance, enhance the antioxidant ability of brain cells, enhance the expression of Bcl-2, and decrease the expression of Bax. Total flavonoids from MDQ can reduce the blood viscosity, enhance the activity of SOD and ATPase in the brain tissue of rats, as well as inhibit the expression of Bax protein and enhance the expression of Bcl-2 protein. It can also relieve the ischemic brain tissue pathological damage in rat models of cerebral ischemic tolerance with blood stasis. It shows that total flavonoids from MDQ can improve the cerebral ischemic tolerance by improving blood circulation, improving energy metabolism, induced enhancement of endogenous antioxidant activity, enhanced anti apoptotic effect, and relieve the injury of nerve cell. This study provides experimental support for the clinical treatment of total flavonoids from MDQ in ischemic cerebrovascular disease.

Acknowledgments

National "Significant New Drug Creation" science and technology major special project (2009ZX09103-324); Henan Province outstanding scientific and technological innovation team (TCJ2014-391); Zhengzhou science and technology innovation team (131PCXTD612).

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