Effect of *Ginkgo biloba* extract on cognitive function and neurotransmitter levels in rats with vascular dementia

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**ABSTRACT**

This study aimed to investigate the effect of *Ginkgo biloba* extract on the cognitive function and neurotransmitter levels in rats with vascular dementia (VD) and its mechanism of action. 50 male Sprague Dawley rats were selected for this study, and the vascular dementia model was established by permanent occlusion of bilateral common carotid arteries of rats. Of which 40 rats were then divided into four groups (n=10): vascular dementia group, low-dose, middle-dose and high-dose *ginkgo biloba* extract groups respectively; another 10 rats were allocated to sham operation group. Rats in low, middle and high dose groups were received 5, 10 and 20 mg/kg/d *Ginkgo biloba* extract via tail vein, respectively, while model group and sham group received an equal volume of normal saline. Morris water maze was used to evaluate the cognitive function of rats. After behavioral observation, these rats were sacrificed for detecting the level of acetylcholine (ACh), dopamine (DA) and 5-hydroxytryptamine (5-HT) in brain tissue.

*Ginkgo biloba* extract can significantly improve the cognitive function of rats with vascular dementia, the mechanism may be correlated with the fact that the extract can obviously increase the levels of ACh, 5-HT and DA, as well as inhibit the activity of AChE.

**Key words:** Cognitive function, *Ginkgo biloba* extract, Neurotransmitter, Vascular dementia.

**INTRODUCTION**

Vascular dementia (VD) refers to a group of clinical syndromes resulting in intelligence and cognitive impairment due to brain tissue damage resulted from cerebrovascular diseases, and it is one of the main types of senile dementia. Moreover, VD is considered as a chronic progressive disease characterized by decline in mental ability or loss in memory, cognition, speech, personality, behavior, judgment, attention and logical reasoning, and it accounts for 35.8%-39% of all the elderly dementia, so it is the second-most-common form of dementia after Alzheimer’s disease (AD) in older adults. This disease not only seriously affects the quality of patients’ life, but also becomes a heavy burden to the family and the community (Chen et al., 2014; Habeych and Castilla-Puentes, 2015). Vascular dementia has been shown to be at least partially preventable and promising in treatment, thus becoming a hot research topic at home and abroad.

In recent years, a large number of studies on the pathogenesis of VD have indicated that the mechanism of VD is related with pathological changes in the cholinergic nervous system, damaged neurons, changes in excitatory amino acids, and the imbalance of free radical metabolism in brain tissue. Moreover, cholinergic nervous system and synaptic abnormalities have close relationship with cognitive activities since the release, transmission and processing of cholinergic neurotransmitter between the synapse between neurons are physiological mechanisms of learning and memory. It has been well-documented that a variety of neurotransmitters in brains of VD patients are changed as well as neural activity and neurotransmitters in the cerebral cortex, hypothalamus, striatum, hippocampus are reduced significantly, especially the decrease in acetylcholine and monoamine neurotransmitter of the cerebral cortex is closely correlated with mental decline of VD patients (Wang et al., 2012; Zhang et al., 2014; Schmitz et al., 2015).

*Ginkgo biloba* extract is considered as active substance extracted from leaves of *Ginkgo biloba*. It exhibits the effects of reducing blood viscosity, improving microcirculation and anti-lipid peroxidation, increasing blood flow, protecting vascular endothelial cells, and ameliorating cerebral artery and peripheral blood flow, as well as protecting brain neurons and improving intelligence. Therefore, *Ginkgo biloba* extract has been widely used in clinical treatment of senile dementia, hypertension, coronary heart disease and arteriosclerosis (Xue and Long, 2012; Wang et al., 2013).

In this study, we investigated the effect of *Ginkgo biloba* extract on the cognitive function and neurotransmitter
levels in VD rats as well as the underlying mechanisms by establishing VD model to provide a theoretical basis for treatment of vascular dementia.

**MATERIALS AND METHODS**

**Experimental animals and grouping:** Sprague Dawley rats, weighting of 180.6±10.23 g, were obtained from Slac Animal Center, Shanghai, China. 50 SD rats were selected for our study; of which 40 were divided into 4 groups (n=10), and they were model group, low dose, middle dose and high dose group, while another 10 rats were allocated to sham operation group. All above rats were anesthetized with 40 g/L pentobarbital sodium by intraperitoneal injection with fasting for 12 h and water deprivation for 4 h before surgery. One rat was fixed with supine position on the disinfection operation table. After depilation in rat neck, skin incision in the middle of the neck was made after 75% alcohol disinfection, and then the bilateral common carotid arteries were isolated, taking care to avoid injury to the cervical sympathetic and vagus nerve, and permanent ligation of bilateral common carotid arteries in rats with 6-0 line was done to block common carotid arterial flow. After carefully stitched, the rats received intramuscular injection of penicillin (20×10^4 U/d) for consecutive 3 days to prevent infection. These rats were incubated at 35°C and housed separately after awaking. Rats in sham operation group only underwent the isolation of bilateral common carotid arteries without occlusion. After successful model construction, low dose, middle dose and high dose groups were administered *Ginkgo biloba* extract via tail vein at the dose rate of 5, 10 and 20 mg/kg/d, respectively, while model group and sham operative group received an equal volume of saline. This study was performed in strict accordance with the recommendatons in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA). Eighth Edition, 2010. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Nanyang the First People’s Hospital.

**Morris water maze test**

The Morris water maze (Zhongshidichuang Science and Technology Development Co., Ltd, Beijing, China) is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory, and in this study, it was conducted as follows.

Platform orientation test: 4 points of water entry for four quadrants in the east, west, south, and north of the pool were fixed, and one point was selected optionally for the platform placement. The rats were daily placed at the 4 water-entering points in the east, south, west and north of the pool in turn. Meanwhile, the time for the rats to find the platform (evasive latency) was recorded during 120 s. If the platform was still not found within 120 s, artificial guidance was conducted for platform arrival with 30 s stay, and the latency was counted as 120 s with continuous testing for 5 days.

Spatial probe test: The platform was removed at the 6th day of experiment, and the rats were placed at one of the 4 water-entering points in the pool. Meanwhile, the frequency of passing through the location of the platform was recorded during 120 s.

**ACh level and AchE activity assays**

All rats were sacrificed after behavioral observation. Brain cortex tissues were immediately taken out and placed on an icy tray, from which 10% tissue homogenate was prepared in normal saline. Above tissue blocks were cut into pieces with ophthalmic scissors as quickly as possible and homogenized for 5 min in 200 ul tissue lysates. Then they were placed on ice for 20 min and centrifuged at 15,000 g for 15 min. Serum supernatant was collected for determination of AChE activity with spectrophotometric method and ACh level with ELISA method in strict accordance with the kit’s instruction (Amyjet Scientific Inc, Wuhan, China).

**DA and 5-TH levels assays**

All rats were sacrificed after behavioral observation. Brain cortex tissues were immediately taken out and placed on an icy tray, from which 0.5 g tissue samples were collected and put into 5 ml pre-cooled acidic N-butanol for preparing tissue homogenate in ice bath with a glass homogenizer. Above tissue homogenate was mixed for 1 min and centrifuged at 15,000 g for 10 min. Serum supernatant was collected and interacted with iodine reagent to form three oxindole compounds and dihydroxy indole compounds in alkaline condition. The fluorescence intensity of 5-HT and DA was measured at 375 nm/325 nm and 360 nm/480 nm with fluorescence spectrophotometer (Haiguang instrument, Beijing, China) according to the related kit instruction (Amyjet Scientific Inc, Wuhan, China). The concentration of 5-HT and DA was calculated according to the standard curve.

**Statistical analysis:** All data were performed using SPSS13.0 software (SPSS Inc, Chicago, IL, USA), and expressed as the mean ± standard deviation. Measurement data was analyzed using t-test while count data was analyzed using chi-square test. P <0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION**

**Comparison of cognitive function of rats in each group:** Platform orientation test showed that the escape latency in low dose group, middle dose group and high dose group was longer when compared with sham operation group (P<0.05); in a dose-dependent manner (Table 1). The results of spatial probe test showed that the frequency of passing through the original platform in low dose group, middle dose...
Table 1: Comparison of cognitive ability of rats in each group (x±s).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>The average of escape latency (s)</th>
<th>Frequency of passing through the location of the platform (Times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation group</td>
<td>10</td>
<td>20.05±6.78</td>
<td>12.78±4.12</td>
</tr>
<tr>
<td>Model group</td>
<td>10</td>
<td>118.76±14.54#</td>
<td>3.78±1.45#</td>
</tr>
<tr>
<td>Low dose group</td>
<td>10</td>
<td>97.12±13.87#*</td>
<td>5.12±2.13#*</td>
</tr>
<tr>
<td>Middle dose group</td>
<td>10</td>
<td>79.57±13.36#*</td>
<td>6.61±2.52#*</td>
</tr>
<tr>
<td>High dose group</td>
<td>10</td>
<td>64.12±10.06#*</td>
<td>7.92±3.03#*</td>
</tr>
</tbody>
</table>

Note: # Compared with sham operation group, P<0.05; * compared with model group, P<0.05.

Fig 1: Comparison of ACh level and AChE activity of rats in each group.

Comparison of Ach level and AchE activity of rats in each group: In model groups, low dose, middle dose and high dose, the level of ACh was markedly decreased (P<0.05) while the activity of AChE was significantly increased when compared with sham operation group (P< 0.05). Moreover, in low dose group, middle dose group and high dose group, the level of ACh was markedly increased (P<0.05) while the activity of AChE was significantly decreased in a dose-independent manner (Fig 1).

Comparison of DA and 5-TH levels of rats in each group

DA and 5-TH levels in model groups, low dose, middle dose and high dose were markedly decreased when compared with sham operated group (P< 0.05). Moreover, DA and 5-TH levels in low dose group, middle dose group and high dose group were significantly increased in a dose-independent manner (Fig 2).

Vascular dementia is a chronic progressive disease, and its clinical symptoms present obvious positioning impairment in the nervous system as well as a series of abnormalities in mental behavior and neuropsychology. In modern society, the incidence of VD increases with population aging, which have serious effect on health and quality of life (Gupta et al., 2015; Zhang et al., 2015). Studies showed that timely treatment of VD in the early stages could achieve reversible recovery, so it presented to be at least partially preventable and promising in treatment (Thomas et al., 2015).

At present, the pathogenesis of VD is not very clear, and most scholars consider that cholinergic nervous system and monoamine neurotransmitters play a crucial role in the development and prognosis of VD. ACh is regarded as an earliest identified neurotransmitter in the cholinergic pathway, which is found to have the closest relation with learning and memory, meanwhile, damage in cholinergic nervous system and it is widely considered to be the vital cause of VD. Modern studies have demonstrated that the central cholinergic system is a major pathway in learning and memory, and the functional decline of the cholinergic pathway in the new cortex or hippocampus is correlated with learning and memory loss of VD patients. Additionally, clinical studies showed that the degree of cognitive deficit in VD patients was related with the decrease in ACh synthesis and increase in AChE activity (Yanf et al., 2014; Li et al., 2014). In this study, ACh level and AChE activity in cortical tissue of VD model rat were detected, and the results showed that the level of ACh in model group was significantly
decreased while the activity of AChE was significantly increased, which were similar to study reported by Zhang et al. (2014).

Monoamine neurotransmitters are one kind of important transmitters in the brain and it is indispensable to memory formation and retention, which mainly include DA and 5-HT. DA can regulate voluntary movement, which plays an important role in improving memory (Wan et al., 2014; Saddoris et al., 2015), while 5-HT, synthesized in the brain nerve cells and important to maintain normal intelligence, whose role is to keep the synaptic connection of cortex and hippocampus and involve in the brain’s cognitive function. When VD occurs, DA and 5-HT systems damage, so the deficiency in DA and 5-HT synthesis can lead to cognitive dysfunction and behavioral disorder of patient with dementia (Gupta and Sharma, 2014). In this study, DA and 5-HT levels in brain cortical tissue of VD model rats were detected, and the results showed that their levels in model group were significantly decreased.

Ginkgo biloba extract has anti-oxidative, free radical scavenging, vasodilative, anti-aging, ischemia-reperfusion injury reduction and memory enhancement effects, of which the possible mechanism is that flavonoids can neutralize oxygen free radicals and prevent blood vessel wall thickening and hardening, thereby improving the supply of oxygen and nutrients in the brain. Therefore, Ginkgo biloba extract has been widely used in the treatment of cardio/cerebrovascular diseases with a significant efficacy (Shao et al., 2003; Ihl et al., 2012). In this study, Ginkgo biloba showed that extract could efficiently shorten the escape latency, increase the frequency of passing through the platform. It significantly increase ACh, DA and 5-TH levels while inhibit AChE activity in a dose-dependent manner, which suggested that Ginkgo biloba extract could significantly improve cognitive function in rats with vascular dementia through increase in ACh, DA and 5-HT synthesis and inhibiting AChE activity.

REFERENCES


