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Effects of galangal extract on cognitive dysfunction and nerve pathological change in rats with diabetic encephalopathy

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ABSTRACT

Objective: To evaluate the effects of galangal extract on cognitive dysfunction and nerve pathological change in rats with diabetic encephalopathy. **Methods:** Sixty male SD rats were given high sugar and fat diet except the control group. Fifty days later, the animals were injected with STZ 30 mg/kg through intraperitoneal to establish type 2 diabetes model. Rats were divided into control group, model group, Metformin group, oxiracetam group, galangal extract high and low dose group. After 4-week administration, Morris water maze was utilized to investigate the effects of different galangal extract on learning and memory ability in rats. After behavioral testing, the blood sugar level was detected. Meanwhile, spectrophotometer was used to measure the superoxide dismutase (SOD) activity and maleic dialdehyde (MDA) content of brain tissue. HE staining was used to observe the morphological changes in the hippocampus. **Results:** Galangal extract can significantly reduce swimming time and swimming distance of diabetic encephalopathy rat model, lower fasting blood glucose while increase body weight. At the same time, SOD activity and MDA content of rat brain were reduced. The morphology of neurons in hippocampus was improved and neuronal nuclear condensation was reduced correspondingly. **Conclusions:** Galangal extract can significantly improve cognitive ability in diabetic rats, reduce hippocampal pathological changes and have some prevention or treatment effects on of diabetes encephalopathy.

1. Introduction

The prevalence of diabetes is increased year by year with the improvement of people's living standards and the impact of aging, cognitive dysfunction resulting from diabetes, a growing concern, and has become one of the important medical issues today[1]. More and more studies show that long-term high glucose can cause central nervous system damage, thereby causing cognitive and learning ability drops, the development of neurological complications of diabetes encephalopathy [diabetes encephalopathy (DE)]. Pathophysiology is currently unclear DE, and brain cognitive dysfunction caused by high blood sugar long-term reasons unclear for such patients it is difficult to provide a suitable means of prevention and drugs[2,3].

Galangal was to be collected contains for the calendar version of "Chinese Pharmacopoeia" from the plant Zingiberaceae alpinia, and medicinal parts of its dried roots ,The main production in China's southeast and southwest provinces, including Fujian, Taiwan, Guangdong, Guangxi, Hainan, Yunnan and other places, its Yield more abundant in which Guangdong and Hainan produced galangal is genuine medicine[4]. It was found to have many pharmacological activities, including strong free radical scavenging and anti-lipid peroxidation, anti-fibrosis, anti-obesity, anti-allergic, anti-microbial and anti-tumor activity and other effects from domestic and foreign scholars in the study of galangal[5,6]. The latest study found galangal water extract and ethanol extract may be directly or indirectly remove free radicals, thereby delaying cell aging, increase the

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concentration of melatonin in the body, reducing the generation of lipid peroxides significantly improve memory impairment in mice hippocampus histological lesions[7]. In addition, galangal extract can exert antioxidant effects in vivo and significantly improve learning and memory ability of aging mice, but the mechanism is not clear and material basis[8]. This study explores the impact of galangal extract on cognitive function of rats with diabetic encephalopathy, also studied the impact on the pathological changes of hippocampal tissue as galangal extract provides experimental support in the prevention and treatment of diabetic encephalopathy application.

2. Materials and methods

2.1. Main reagents, drugs and equipment

Galangal extract from Hainan Medical College Department of Pharmacognosy; Streptozotocin (STZ) purchased from Sigma; Metformin hydrochloride tablets purchased from Beijing Sihuan Pharmaceutical Co., Ltd. Batch number: 150601; Oxiracetam capsule purchased from Ouyi CSPC Pharmaceutical Co., Ltd. Batch number: 20150802; Morris water maze device system purchased from Chengdu Thai Meng Technology Co., Ltd; Superoxide dismutase (SOD) and maleic dialdehyde (MDA) purchased from Nanjing Jiancheng Institute of Biotechnology.

2.2. Preparation and administration of animal model

SD rats, male, cleaning stage, weight: 250-300 g, purchased from Changsha Tian Qin Biotechnology Co., Ltd. Animal Certificate of Conformity: SCXK 2009-0012. All animals were maintained 12 h circadian rhythms at housed separately by free drinking water ingestion, room temperature: (22 ± 1)℃.

Its water maze training was performed twice in rat adaptive feeding one week, and the cognitive ability was selected to meet the requirements of rat reserved for formal experiment. Rats were given high fat high sugar diet from Changsha Tian Qin Biotechnology Co., Ltd except the control group rats were given basic feed (Basal diet containing 60%, 20% sugar, 16% fat and 4% cholesterol). Test rats had intraperitoneal injection at a concentration of 30 mg/kg of STZ solution according to body weight by fed 50 d later. Rat blood glucose concentration were from 0.2 mL of rat tail vein blood at automatic blood glucose meter blood glucose concentration for three days late. Successful model standard blood glucose concentration determination was 16.7 mmol /L, and insulin resistance in rats. Cognitive function by the water maze test showed that the previous modeling STZ was significantly impaired compared to that of rats diabetic encephalopathy successful model. These 50 successful modeling rats were randomly divided into model group, oxiracetam group (50 mg/kg), metformin group (100 mg/kg) and the high, low dose group of galangal extract groups, also control group. All treatments were given through oral once daily, and the control group and model group with distilled water. Follow-up test was performed after four weeks of continuous administration wherein after administration of the first 14 d and 21 d rat blood glucose levels and body mass detection.

2.3. Experiment of Morris water maze[9]

Rats received morning and afternoon training two times in the last 1 h after gavage, and every time the rats were toward pool wall from four different directions of fixed sites. Water temperature was 28 ℃ at room temperature. The camera continuously recorded the time required to find the platform within 90 s as the escape latency, while the software recorded swim distance. The time recorded as 90 s if the platform was not found within the 90 s, then the rat was placed on artificial platform resting out of the pool after 20 s. Two determinations interval was at least 30min, continuous training 4 d after removal of the platform in the first five days. The rats were facing the wall, point into the water was randomly selected into the water, and cameras recorded the number of times across the platform within 90 s and the cumulative residence time in the target quadrant.

2.4. Brain histopathology

The whole brains of rats in decapitation were taken at half an hour after the end of the Morris water maze test. Surface blood soaked with cold saline brain tissue was fully removed three times. The brain tissue was trimmed with a razor blade along the sagittal plane into two halves after dry filter paper. Half of the brain was stained with 10 mL 4% paraformaldehyde fixed 24 h while hippocampus-related pathological changes of hippocampus parallel slices was observed after paraffin-embedded under Olympus microscope. The other half of the brain was used for measuring other parameters.

2.5. Brain tissue SOD activity and MDA content in rat

The other half of the brain tissue was taken after the Morris water maze test ends. Saline was added at 4 ℃ pre-cooling, 10% brain tissue at weight/volume ratio of 1: 9 was centrifuged at 4 ℃, 3 000 r/min for 20 min. The supernatant was transferred slowly to an ice-bath and sample preparation was performed by Nanjing Jiancheng Biological Engineering Company. Specification standard curve of absorbance measurement, SOD and MDA content were calculated.

2.6. Statistical analysis

All data were processed by SPSS 13.0 statistical software for analysis and the results are expressed as mean ± sd. After the homogeneity, variance between the detection of qualified groups was compared by single factor analysis of variance, P <0.05 was considered as significant difference.

3. Result

3.1. Effect of galangal extracts on diabetic rats Morris water maze encephalopathy swimming time and swimming distance

It showed that no significant difference was observed among the groups in swimming time and swimming distance at day 1. In model group swimming time and distance are significantly were increased from the 3rd day. Exploration swimming time and distance were significantly reduced compared to the positive drug group and model group oxiracetam (Table 1 &2).

3.2. Effect of galangal extract on blood glucose and body weight in diabetic encephalopathy

Body weight was significantly decreased and blood glucose levels of model group were significantly increased significant increase compared with control group; body weight of galangal alcohol extract low concentration group was increased significantly compared with the model group and metformin group and blood glucose level was significantly decreased. Oxiracetam group had no significant effect on blood glucose and body weight (Table 3&4).

3.3. Effect of galangal extracts of homogenates SOD activity and MDA content in brain tissue of rats with diabetic encephalopathy

It could significantly increase SOD activity and decrease MDA content in rat brain tissue of homogenates oxiracetam and galangal extract alcohol group as compared with the model group (Table 4), and the effect of galangal alcohol extract low concentration group

Table 1
Effect of water maze test swim time galangal extracts on diabetic encephalopathy rats.

Groups	Dose(mg/kg)	Swim time (s)			
		Day1	Day2	Day3	Day4
Control group		80.31 ± 19.34	67.50 ± 23.79	49.74 ± 18.40	31.02 ± 18.69
Model group		91.47 ± 21.45	84.32 ± 17.50	77.21 ± 32.22$^{\#}$	65.08 ± 14.37$^{\#\#}$
Oxiracetam group	50	84.61 ± 14.70	75.22 ± 12.65	62.07 ± 24.92*	38.09 ± 23.10**
Metformin group	100	87.19 ± 10.49	73.25 ± 24.75	66.28 ± 19.80	42.92 ± 29.50*
Galangal alcohol extract group	100	82.41 ± 17.76	72.40 ± 19.94	60.56 ± 23.32*	44.18 ± 12.20*
Galangal alcohol extract group	200	79.55 ± 16.91	64.47 ± 26.05*	55.92 ± 31.81*	33.19 ± 19.52**

Note: Compared with the control group, $^{\#}$ P<0.05, $^{\#\#}$ P<0.01; compared with model group, * P<0.05, ** P<0.01.

Table 2
Effect of water maze test swim distance galangal extracts on diabetic encephalopathy rats.

Groups	Dose(mg/kg)	Swim distance (m)			
		Day1	Day2	Day3	Day4
Control group		18.34 ± 1.94	13.19 ± 1.23	7.74 ± 1.25	5.21 ± 1.03
Model group		21.09 ± 5.04	22.74 ± 3.12$^{\#}$	19.30 ± 2.30$^{\#\#}$	20.56 ± 5.71$^{\#\#}$
Oxiracetam group	50	19.32 ± 3.16	16.06 ± 4.39	12.13 ± 5.27*	8.56 ± 7.04**
Metformin group	100	20.47 ± 4.01	19.71 ± 3.64	17.13 ± 4.25	18.26 ± 6.34
Galangal alcohol extract group	100	18.54 ± 2.89	14.51 ± 5.59	12.42 ± 5.87*	8.93 ± 4.90**
Galangal alcohol extract group	200	18.47 ± 3.35	11.75 ± 6.04*	9.62 ± 3.58**	6.17 ± 4.52**

Note: Compared with the control group, $^{\#}$ P <0.05, $^{\#\#}$ P<0.01; compared with model group, * P<0.05, ** P<0.01.

Table 3
Effect of galangal extract on blood glucose and body weight in diabetic encephalopathy.

Groups	Dose(mg/kg)	Body weight (g)		Blood glucose (mmol/L)	
		14 d	21 d	14 d	21 d
Control group		423.50 ± 36.10	461.70 ± 27.40	5.14 ± 0.73	4.82 ± 0.60
Model group		341.05 ± 29.82$^{\#\#}$	357.30 ± 41.67$^{\#\#}$	23.16 ± 1.77$^{\#\#}$	25.14 ± 3.79$^{\#\#}$
Oxiracetam group	50	351.82 ± 45.26	355.60 ± 41.06	21.34 ± 3.16	24.53 ± 3.71
Metformin group	100	401.65 ± 38.03**	406.30 ± 51.42**	13.61 ± 1.85**	12.26 ± 1.61**
Galangal alcohol extract group	100	378.91 ± 46.50	392.30 ± 46.42*	17.36 ± 1.48*	18.47 ± 2.32**
Galangal alcohol extract group	200	413.71 ± 52.06**	437.60 ± 63.45**	14.71 ± 1.68**	11.06 ± 1.43**

Note: Compared with the control group, $^{\#}$ P<0.05, $^{\#\#}$ P<0.01; compared with model group, * P<0.05, ** P<0.01.

was better than group oxiracetam.

Table 4

Effect of galangal extracts of homogenates SOD activity and MDA content in brain tissue of rats with diabetic encephalopathy.

Groups	Dose (mg/kg)	SOD activity （U/mgpr）	MDA content （nmol/mgpr）
Control group		93.64 ± 11.33	57.38 ± 8.16
Model group		42.51 ± 9.35$^{\#\#}$	119.03 ± 26.70$^{\#\#}$
Oxiracetam group	50	63.73 ± 14.04**	83.45 ± 17.95*
Metformin group	100	47.08 ± 10.66	104.75 ± 22.30
Galangal alcohol extract group	100	83.25 ± 18.55**	74.61 ± 11.72*
Galangal alcohol extract group	200	103.58 ± 26.74**	68.40 ± 17.94**

Note: Compared with the control group, $^{\#}$ P<0.05, $^{\#\#}$ P<0.01; compared with model group, * P<0.05, ** P<0.01.

3.4. Effect of galangal extract on rat hippocampal morphology of diabetic encephalopathy

Compared with the control group, the density of neurons in hippocampal CA1 region of rats in model group was decreased, accompanied by some of the cells degeneration and necrosis; the cell gap was increases. Cell volume expansion, cytoplasm stained axons disappearance, apparent nuclear condensation, nuclear envelope membrane fuzzy boundaries appeared. Oxiracetam positive drug and alcohol galanga extract low-dose group were reversed to varying degrees in CA1 pyramidal cell morphology diabetic encephalopathy hippocampus changes (Figure 1).

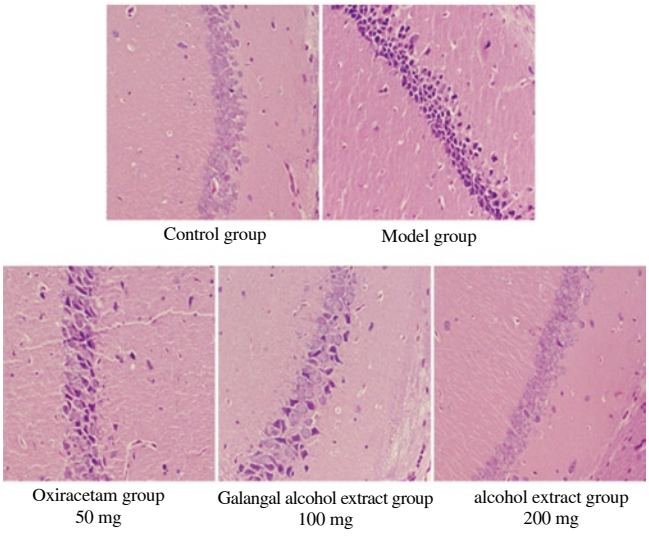

Figure 1. Effect of alcohol extract of galangal encephalopathy induced diabetic rat hippocampal tissue morphology (HE staining).

4. Discussion

With the aging of population growth, the incidence of diabetes in our country continues to rise, more and more younger age of onset. Cognitive impairment caused by diabetic encephalopathy has become a new hotspot for diabetic nephropathy and diabetic encephalopathy research is a growing concern from many complications of diabetes. People with diabetes encephalopathy yet to establish an effective prevention strategy since the pathogenesis is unknown. Diabetic encephalopathy is one of the main factors that eventually lead to dementia that mainly for the lack of memory on the clinical, emotional disorders and corresponding internal morphological and physiological changes, leading to patients with neuropsychiatric symptoms. It is generally believed that blood sugar disorder caused by brain endothelial cells and nerve cells from oxidative stress; it can directly damage the brain microcirculation and neurovascular unit. Neurotrophic factor supply deficiency cerebral blood barrier micro structural changes and pathogenesis of degeneration of cholinergic neurons are involved in the pathological process[10]. Modern pathology and pharmacology studies have shown that development of diabetic encephalopathy involves a number of functional cells in vascular endothelial cells, neurons, glial cells, cells involved in inflammation. Vieira *et al* proposed for the complex pathogenesis of diabetic encephalopathy, prevention and treatment required for the onset of multiple pathways while Select multi-link, multi-target therapeutic agents to intervene[3]. This medicine multi-component, multi-target effects characteristic coincides.

Hainan galangal is genuine medicine. Recent studies have found that many pharmacological activity including strong free radical scavenging and significant inhibition of lipid peroxidation, anti-fibrotic, anti-obesity, anti-allergic and anti-tumor activity[11]. Zhao *et al* that also find galangal water extract and ethanol extract can remove free radicals directly or indirectly, thereby delaying cell aging, increase the concentration of melatonin in the body, reducing the generation of lipid peroxides and significant improvement in memory impairment in mice hippocampus histological lesions[7]. Therefore, galangal extract investigate their cognitive function in diabetic rats encephalopathy and the pathological cerebral in this study.

Water maze test is one of the more recognized neuro-cognitive function and memory improve outcomes assessment tools[12,13]. The results of this study show galanga extract for oral administration can significantly reduce the escape latency and shorten the swimming distance to explore and increase the number of crossing the target quadrant virtual platform residence time. This study show galanga extract can improve memory in rats and significant improvement in symptoms of cognitive dysfunction, the result is better than the positive drug group oxiracetam.

The study did not model rats group weight gain, shiny coat, food and water excretion and urine volume normal within the normal range. Gradually showing diabetes unique "three high and one low" performance with the extension of the model group modeling time that is an increase in water intake feeding a significant increase in urine output, decreased weight, and hair color brown, reduce the spontaneous activity; Morris water maze test display accompanied by significant cognitive dysfunction and swimming time and distance is extended. Animal models simulate the clinical

manifestations of diabetic encephalopathy. Moreover, blood glucose test results showed that the model group was significantly higher than the control group, and the galangal low-dose group could significantly improve the "three high and one low" symptoms. Model group increased weight and lower blood sugar. This study show galanga extract in STZ-induced diabetic rats has a therapeutic effect. And hyperglycemia-induced oxidative stress and inflammation are important factors for diabetes complications start. Thus its can effectively lower blood sugar and reduce the delay the development of diabetic encephalopathy, thereby improving the various complications of diabetes. This may be one of the mechanisms galanga extract improve brain neurological changes.

Homogenates of rat brain and hippocampal morphology is further evidence that the ethanol extract of galangal low concentration group could significantly increase in rat brain homogenates SOD activity and decrease MDA content meanwhile varying degrees improved morphology CA1 pyramidal cells in the hippocampus of rats diabetic encephalopathy. These results strongly suggest that galangal extract may by its hypoglycemic effect and in vivo antioxidant effect,and the function of hippocampal damage and cognitive pathological changes of diabetic encephalopathy significant improvement. And worth further explore the mechanism and the development of an active ingredient.

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