

Effects of *Astragalus* polysaccharides on memory impairment in a diabetic rat model

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Objective: *Astragalus* polysaccharides (APS) are active constituents of *Astragalus membranaceus*. In this study, we aimed to investigate the effects of APS on memory impairment in a diabetic rat model and their mechanisms.

Methods: A diabetic model was established in 50 male Wistar rats with streptozotocin intraperitoneal injection. A blood glucose level higher than 16.7 mmol/L obtained 72 hours after the injection was regarded as a successful diabetic model. The modeled rats were divided into model group, high, medium, and low doses of APS, and piracetam groups (positive control). A group of ten rats without streptozotocin-induced diabetes were used as a normal control. After respective consecutive 8-week treatments, the levels of blood fasting plasma glucose, insulin, hemoglobin A1c, memory performance, hippocampal malondialdehyde, and superoxide dismutase were determined.

Results: After the 8-week APS treatment, serum fasting plasma glucose, hemoglobin A1c, and insulin levels were decreased compared with those of the model group ($P < 0.05$). Importantly, memory impairment in the diabetic model was reversed by APS treatments. In addition, hippocampal malondialdehyde concentration was lowered, whereas that of superoxide dismutase was higher after APS treatments.

Conclusion: APS are important active components responsible for memory improvement in rats with streptozotocin-induced diabetes. The potential mechanism of action is associated with the effects of APS on glucose and lipid metabolism, and antioxidative and insulin resistance. APS are constituents of *A. membranaceus* that are potential candidate therapeutic agents for the treatment of memory deficit in diabetes.

Keywords: diabetic, *Astragalus* polysaccharides, memory impairment, MDA, SOD

Introduction

Diabetes mellitus is the most common type of metabolic disorder worldwide, and its incidence is increasing annually. In 2014, it was estimated that 8.3% of adults globally have diabetes (*International Diabetes Federation Atlas*, 6th edition).¹ Diabetes and insulin resistance are associated with changes in the central nervous system and the development of cognitive and memory impairments. The rising prevalence of diabetes and its earlier onset indicate that diabetes-related cognitive and memory dysfunction may increase substantially in the near future, causing tremendous socioeconomic burdens. Cognitive dysfunction affects the quality of life of diabetic patients and augments the risk of dementia.^{2,3} Therefore, the elucidation of the mechanisms of memory deficit and discovery of drugs with high therapeutic effects are urgently required in the treatment of diabetes.

The processes of learning and memory are related to the activities of a number of biological structures. The hippocampus is important for memory formation.⁴ Although

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the mechanisms underlying memory deficit in diabetes are not well understood, there are some implications from previous research. First, deviant sleep due to impaired glucose metabolism is shown to produce memory deficits in patients with diabetes mellitus.⁵ Second, chronic hyperglycemia in rats was reported to result in a significant loss of cortical neurons, which may impair cognition.⁶ In addition, neurotransmitter systems, such as gamma-aminobutyric acid, and cholinergic systems involved in learning and memory are affected.⁷ The findings of animal studies also indicate that chronic hyperglycemia induced by streptozotocin (STZ) decreases the synthesis and release of acetylcholine in rat brain.⁸

Herbal medicines have long been an attractive option in the treatment of diabetes. *Astragalus mongholicus* (Huangqi) was documented to reduce blood glucose level in diabetic patients.^{9–11} More importantly, it has the ability to ameliorate brain functions in a diabetic model.¹² However, the exact ingredients involved in the effect of *A. mongholicus* are not identified. The active constituents of the plant include polysaccharides, saponins, flavonoids, L-arginine, and L-canavanine.^{9,13} Among these, *Astragalus* polysaccharides (APS) have been most widely investigated, mainly with respect to their immunopotentiating properties, ability to counteract the side effects of chemotherapeutic drugs, and anticancer activities.^{13–15} In this study, APS were applied in a STZ-induced diabetic rat model to explore their potential protective mechanisms.

Materials and methods

Diabetic model and treatment

Fifty-four SPF-grade male Wistar rats (weighing 260–380 g, Application No: 20140038) were obtained from the Animal Center of Shandong University and housed in a 12-hour light–dark cycle with food and water ad libitum under controlled temperature ($23^{\circ}\text{C}\pm 2^{\circ}\text{C}$) and humidity ($55\%\pm 5\%$). The animal experiments were reviewed and approved by the Animal Care and Use Committee of the First Hospital of Hebei Medical University and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals. All were subjected to fasting for 16 hours. Then, 50 of them were intraperitoneally injected once with 10% STZ (50 mg/kg). STZ was dissolved in citrate buffer with pH adjusted to a value of 4.5. Seventy-two hours after injection, blood was drawn from the tail vein, and blood glucose levels were determined. The diabetic models were regarded as successful if their blood glucose levels were higher than 16.7 mmol/L. The animals with diabetes were divided randomly into five groups ($n=9/\text{group}$): model group,

piracetam group, group treated with a high-dose of APS (800 mg/kg/day), group treated with a medium-dose of APS (400 mg/kg/day), and group treated with a low-dose of APS (200 mg/kg/day). APS and piracetam were administered orally for 8 consecutive weeks. Piracetam (Batch No: H12020667, Tianjing Jinshi Pharmacy Co. Ltd., Tianjin, People's Republic of China) was applied as a positive control at a dose of 500 mg/kg/day. APS (Xi'an Ruilin Biotech, Xi'an, People's Republic of China) were prepared as suspension liquid. A group of ten rats without STZ injection was set as a normal control.

FPG, HbA1c, and insulin measurements

The levels of serum blood glucose were measured every 4 weeks. Twenty-four hours after the last drug treatment, vein blood was collected for fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and insulin measurements (Sigma-Aldrich Co., St Louis, MO, USA).

Morris water maze

Animals were tested by Morris water maze 8 weeks after the treatments. At the beginning of the place navigation task, rats were placed in a pool without a platform, allowing them to swim for 5 minutes and familiarize with the environment. Tests were carried out at a fixed time every day for 5 days, and there were four training sections during each time period (120 seconds). Rats were placed into the pool facing the wall at any of the four starting points. Video camera tracking system recorded the time necessary for rats to find the platforms (escape latency) and their swimming routes. During the four training sections, rats were placed into the pool at four different starting points (different quadrants). If the animals did not find the platforms within 120 seconds during the Morris water maze test, the latency was assigned 120 seconds. Then, the rats were positioned onto the platforms, allowing a 15 second rest before the next training section. The average value of latency during four training sections performed every day was recorded as the study result of that day. On the sixth day of spatial probe, the platform was removed, and rats were placed into water at a randomly selected, but the same for all animals, starting point. The time interval during which rats stayed in the quadrant with the platform was recorded.

MDA and SOD measurements

After the behavioral measurements, the rats were decapitated. Hippocampus was isolated for malondialdehyde (MDA) and superoxide dismutase (SOD) tests. Hippocampal

MDA and SOD activities were detected by assay kits (Sigma-Aldrich Co.).

Statistical analysis

All data were presented as mean \pm standard deviation. Differences among three or more groups were compared by one-way analysis of variance, followed by Bonferroni post hoc testing for multiple comparisons. Differences between two groups were assessed by the Student's *t*-test. *P*-values of 0.05 or less were regarded as significant.

Results

APS decreased FPG and HbA1c levels in the diabetic model

As compared with the normal control, the induction of diabetes resulted in elevated blood FPG and HbA1c levels (Table 1). After treatments for 8 weeks, FPG level was significantly decreased in the groups with the three doses of APS and the piracetam group ($P < 0.05$ vs model).

HbA1c level was also increased in the diabetic model group compared with the normal control. APS or piracetam treatments attenuated the elevated HbA1c concentrations in the diabetic model ($P < 0.05$ vs model) (Table 1).

APS reduced serum insulin level

After diabetic modeling, the insulin level was considerably downregulated when compared with the normal control. The treatment with APS at all three doses or with piracetam significantly elevated the insulin level ($P < 0.05$ vs model) (Table 2).

APS ameliorated the memory impairment in the diabetic model

Compared with the normal control, the latency to find the platform was significantly increased in the diabetic model

group ($P < 0.05$). However, the treatment with APS at all three doses or with piracetam restored the latency ($P < 0.05$ vs model) (Table 3). On the sixth day, after removal of the platform, the rats in the diabetic model group spent less time in the target quadrant compared with the normal control ($P < 0.05$ vs control). However, the treatment with APS at all three doses or with piracetam apparently restored the memory deficit ($P < 0.05$ vs model) (Table 4).

APS elevated SOD and MDA levels in the diabetic model

After modeling, hippocampal SOD activity was significantly decreased, whereas MDA level was increased. After the treatments, the SOD concentration was substantially augmented in the treatments with the three doses of APS and with piracetam. By contrast, the MDA level was decreased after the treatments with APS or piracetam ($P < 0.05$ vs model) (Table 5).

Discussion

Huangqi is a common herbal medicine widely applied in different prescriptions for therapy of diseases, especially in Korea and People's Republic of China. As documented, Huangqi possesses antioxidative and cardiovascular ameliorating activities.^{16,17} The herb has been utilized to treat cerebral ischemia^{18,19} and maintain the integrity of the blood-brain barrier.²⁰ Its application was also effective in the therapy of diabetic nephropathy, and by ameliorating the function of kidneys, it caused a reduction in urine protein excretion.^{21,22} Clinically, Huangqi was reported to reverse memory impairment in diabetic patients.^{23–25} These findings indicate the cerebral protection promoted by the drug. Mechanically, it is likely that Huangqi exerts its neuroprotective action through its anti-inflammatory, antioxidative, and antiapoptotic activities.²⁶ Nevertheless, the exact ingredients

Table 1 FPG and HbA1c levels in different rat groups (mean \pm SD, $n=10$)

Groups	FPG (mmol/L)			HbA1c (%)
	0 w	4 w	8 w	
Control	6.66 \pm 0.19	6.77 \pm 0.26	6.90 \pm 0.49	4.55 \pm 1.41
DM	20.78 \pm 2.22*	21.61 \pm 2.21*	20.11 \pm 1.22*	9.13 \pm 1.12*
Piracetam	21.69 \pm 2.51*	18.33 \pm 1.09*	16.69 \pm 0.89*	7.12 \pm 1.09*
Low dose of APS	21.68 \pm 3.07*	19.89 \pm 2.09*	17.33 \pm 2.48*□△	8.56 \pm 1.44*□△
Medium dose of APS	23.51 \pm 4.88*	20.69 \pm 2.89*	16.87 \pm 1.57*□△	7.78 \pm 1.21*□△
High dose of APS	24.56 \pm 5.12*	20.22 \pm 2.45*	16.13 \pm 2.22*□△	7.23 \pm 1.15*□△

Notes: * $P=0.0014 < 0.05$ compared with the control; □ $P=0.0000 < 0.05$ compared with the model group; △ $P=0.0000 < 0.05$ compared with the piracetam group.

Abbreviations: APS, *Astragalus* polysaccharides; DM, diabetes mellitus; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; SD, standard deviation; w, weeks.

Table 2 Serum insulin levels in different groups (mean \pm SD, n=10)

Groups	Insulin level/(μ U/mL)
Control	17.23 \pm 7.32
DM	12.03 \pm 2.39*
Piracetam	14.44 \pm 5.29* \square
Low dose of APS	17.67 \pm 6.56* \square Δ
Medium dose of APS	16.55 \pm 6.78* \square Δ
High dose of APS	14.22 \pm 2.19* \square Δ

Notes: * $P=0.0014<0.05$ compared with the control; $\square P=0.0000<0.05$ compared with the model group; $\Delta P=0.0000<0.05$ compared with the piracetam group.

Abbreviations: APS, *Astragalus polysaccharides*; DM, diabetes mellitus; SD, standard deviation.

responsible for the protection are not identified. In this study, we evidenced that APS, constituting one of the major components, ameliorated the memory impairment in diabetic model rats. These data implicate the action of these compounds for inducing the neuroprotective effects of the herb.

APS are the most important components in *Astragalus membranaceus* and have been clinically applied in the treatment of cancer as an adjunctive medicine enhancing immune activity.¹⁵ APS are also effective for the treatment of diabetic nephropathy, possibly through ameliorating glucose metabolism and decreasing the levels of transform growth factor beta 1.²⁷ In addition, APS are involved in oxidative stress elimination and insulin resistance.²⁸ Based upon this evidence, APS present a high potential to reverse memory deficit in diabetic animal models.

In our study, diabetic rats underwent 8-week APS treatment. The latency to find the platform was significantly shortened after the treatment. Meanwhile, serum FPG and HbA1c levels were remarkably decreased. These data implicate that glucose metabolism is altered after a treatment with APS. APS also decreased the insulin level, indicating the influence of APS on insulin resistance. In addition, we

Table 3 Latencies to find the platform in different groups (mean \pm SD, n=10)

Groups	Latency (s)	
	Before treatment	Posttreatment
Control	45.44 \pm 2.45	47.21 \pm 2.33
DM	83.11 \pm 5.13*	86.35 \pm 4.77* \square
Piracetam	74.32 \pm 4.34*	44.23 \pm 4.54* \square
Low dose of APS	71.11 \pm 2.37*	43.66 \pm 4.55* \square
Medium dose of APS	73.67 \pm 4.24*	42.45 \pm 4.23* \square
High dose of APS	70.56 \pm 3.33*	40.34 \pm 3.21* \square

Notes: * $P=0.0014<0.05$ compared with the control; $\square P=0.0000<0.05$ compared with the model group.

Abbreviations: APS, *Astragalus polysaccharides*; DM, diabetes mellitus; SD, standard deviation.

Table 4 The time spent in the target quadrant by the different groups (mean \pm SD, n=10)

Groups	Before treatment (%)	Posttreatment (%)
Control	38.8 \pm 1.4	37.3 \pm 1.9
DM	20.6 \pm 1.3*	21.2 \pm 2.1* \square
Piracetam	20.3 \pm 1.2*	33.4 \pm 3.7* \square
Low dose of APS	20.8 \pm 1.3*	31.2 \pm 1.8* \square
Medium dose of APS	20.1 \pm 1.5*	28.2 \pm 2.8* \square
High dose of APS	20.2 \pm 1.3*	34.3 \pm 2.7* \square

Notes: * $P=0.0014<0.05$ compared with the control; $\square P=0.0000<0.05$ compared with the model group.

Abbreviations: APS, *Astragalus polysaccharides*; DM, diabetes mellitus; SD, standard deviation.

measured the hippocampal MDA and SOD levels. MDA is an important biomarker of lipid metabolism.²⁹ The decrease of MDA after the APS treatment indicated that the regulation of the lipid metabolism of the experimental animals was improved after the APS treatment. SOD is a marker of antioxidative ability.³⁰ The increase in SOD concentrations revealed that the antioxidative ability was increased after the application of APS.

We explored the impact of APS treatment on the recovery of memory and its underlying mechanisms. However, there are a large number of other bioactive components present in the herb. More clinical studies are required to classify the active components involved in the neuroprotective activities of *A. membranaceus* in diabetes.

Conclusion

Through this study, we provide evidence that APS are important active components responsible for the improvement of memory impairment caused by diabetes. The potential mechanism of action is associated with the effects of APS on glucose and lipid metabolism, their antioxidative properties, and influence on insulin resistance. APS are potential candidate therapeutic agents for the treatment of memory deficit in diabetes.

Table 5 Hippocampal SOD and MDA levels in the different groups (mean \pm SD, n=10)

Groups	SOD (U/mg protein)	MDA (mmol/mg protein)
Control	10.22 \pm 0.19	4.77 \pm 1.45
DM	6.67 \pm 0.29*	12.56 \pm 1.15*
Piracetam	7.78 \pm 0.23* \square	9.33 \pm 0.73* \square
Low dose of APS	6.65 \pm 0.33* \square Δ	11.41 \pm 1.47* \square Δ
Medium dose of APS	7.13 \pm 0.30* \square Δ	10.45 \pm 1.09* \square Δ
High dose of APS	8.22 \pm 0.31* \square Δ	10.41 \pm 0.48* \square Δ

Notes: * $P=0.0014<0.05$ compared with the control; $\square P=0.0000<0.05$ compared with the model group; $\Delta P=0.0000<0.05$ compared with the piracetam group.

Abbreviations: APS, *Astragalus polysaccharides*; DM, diabetes mellitus; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase.

Disclosure

The authors report no conflicts of interest in this work.

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