Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **32** (8): 1152-7 (2013)

Regular article
Received: May 7, 2013
Revised version: July 24, 2013
Accepted: July 25, 2013

Protection Effect of an Oligosaccharide Extracted from Radix Morindae Officinalis on Senile Dementia in Rats

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***SUMMARY*. Bajijiasu is an oligosaccharide extracted from the traditional Chinese medicine Radix Morindae Officinalis (*Morinda officinalis* F.C. How) used for disease about intelligence and memory. The effects of Bajijiasu on learning and non-cognitive disturbances have been confirmed. However, its potential to protect against Alzheimer's disease was not clearly studied. In this work, we want to study the effects of bajijiasu in a rat model of senile dementia and the mechanism. Senile dementia was modeled in rats and bajijiasu was administered for 60 consecutive days by three routes: oral capsules, water solution by gavage, and extracts by gavage. Learning and memory function was assessed with the Morris water maze. We measured brain tissue contents of acetylcholinesterase (AChE), superoxide dismutase (SOD), and malondialdehyde (MDA). Basal forebrain, hippocampal, and cerebral cortex neurons were quantitatively assessed with hematoxylin-eosin (HE) staining and light microscopy. Compared with the model group, rats that received bajijiasu capsules exhibited reduced escape latency and significantly greater SOD activity and reduced MDA content in brain tissue in a dose-dependent manner. All of these measurements were significantly different ($P<0.05$) in the 240 mg/kg group. Microscopy indicated that except for the 30 mg/kg group, all capsule doses increased the number of neurons in the basal forebrain, hippocampus, and cerebral cortex. Bajijiasu can improve learning and memory abilities, increase SOD activity, reduce MDA levels, and increase neuron quantities in the brain tissue of a rat model of senile dementia induced by D-galactose and aluminum trichloride. These effects may be due to improved brain metabolism.**

INTRODUCTION

Senile dementia, also called Alzheimer's disease (AD), is an age-associated neurological disease. The major symptom of AD is short-term memory dysfunction, and the primary pathological changes are senile plaques of amyloid beta, neurofibrillary tangles of hyperphosphorylated tau, specific neurotransmitter deficiencies, and neuronal reductions in the cortex and hippocampus. Between 2000 and 2008, deaths associated with cardiac diseases, stroke, and prostate cancer decreased by 13, 20, and 8%, respectively, but the death rate due to AD increased by 66%. Thus, AD poses serious risks to both human health and quality of life [1,2].

Aging is the strongest risk factor associated with AD, but the underlying mechanisms of AD are not fully understood yet, and no pharmacological measures have proven effective in the clinical setting. An important topic in the AD field is the investigation of disease pathogenesis, with the ultimate goal of developing effective preventative and therapeutic agents [3].

Bajijiasu (Fig. 1) is an oligosaccharide extracted from the traditional Chinese medicine Radix Morindae Officinalis (*Morinda officinalis*

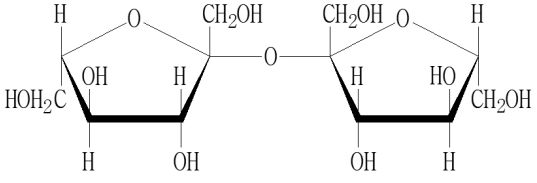

Figure 1. The structure of bajijiasu.

KEY WORDS: AChE, bajijiasu, Brain metabolism, MDA, Radix Morindae Officinalis, Senile dementia, SOD.

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ISSN 0326-2383

F.C. How) and was previously called bajisu [4]. Pharmacological experiments demonstrated that bajijiasu can protect anoxic brain tissue in a senile rat model and improve the memory function. The mechanism is believed to involve nitric oxide (NO) [5]; bajijiasu increases NO and glucose levels and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, and decreases the production and accumulation of lipid peroxides (LPO) and lipofuscin [6]. Tan *et al.* [7] tested rats using the Morris water maze and found that bajijiasu shortened escape latency, prolonged primary platform swimming time and 40 cm ring swimming time, and increased the percentage of primary platform swimming and long-term potentiation (LTP) in hippocampal slices in a dose-dependent manner. Liang *et al.* [8] found that bajijiasu significantly increased the number and area of synapses and synaptic vesicle release in the frontal lobe of aged rats, suggesting that the substance enhances synaptic plasticity, promotes neurotransmitter synthesis, and improves morphological structures responsible for learning and memory. In this study, we investigated the effects of bajijiasu in an AD rat model [9] to preliminarily explore the mechanism and its feasibility in treating AD.

Bajijiasu is easily damaged by dilute hydrochloric acid and artificial gastric fluid *in vitro,* but it is stable in pH 6.8 phosphate buffer and artificial intestinal juice [10]. Compound absorption is similar in all rat intestinal segments ($P > 0.05$) [11]. Therefore, in this study, bajijiasu was loaded into the miniature enteric capsules and administrated orally to ensure that bajijiasu would not be damaged and would be able to exert its effects. We also dissolved or resuspended the substance in water for gavage administration and compared the efficacies of bajijiasu by different routes.

MATERIAL AND METHODS

Materials

In this study, the following devices were used: a YP3001N electronic balance scale with 0.01 g precision (Shanghai Precision & Scientific Instrument Co., Ltd., China), a Morris water maze analysis system DigBehv (Shanghai Jiliang Software Technology Co., Ltd., China), a UV-2800 ultraviolet-visible spectrophotometer (Unicol Instrument Co., Ltd., Shanghai, China), an ELX800 automated enzyme-labeling instrument (BIO-TEX, USA), and a BX41 fluorescence imaging microscope (Olympus, Japan).

Reagents

The following reagents were used: acetylcholinesterase (AChE) kits (batch number: 20091023; Nanjing Jiancheng Bioengineering Institute, Nanjing, China), malondialdehyde (MDA) kits (batch number: 20091023; Nanjing Jiancheng Bioengineering Institute, China), SOD kits (batch number: 20100112; Nanjing Jiancheng Bioengineering Institute, China), piracetam tablets (batch number: 081004; Guangdong Zhongsheng Pharmacy, China), raw Bajijiasu with 96.49% purity confirmed by high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD) (batch number: 20090921, self made), D-galactose (batch number: CAS#:59-23-24; MBCHEM, China), aluminum trichloride (batch number: 20071102; Tianjin Fuchen Chemical Reagents Factory, China), sterilized saline (batch number: 09052805; Guangdong Lital Pharmaceutical Co., Ltd), and micro enteric capsules (batch number: 20091009; Guangdong Qiqiang Pharmaceutical Factory, China). Bajitian medicinal materials were purchased in September 2009 from Gaoliang Town, Deqing City, Gudong Province and certified by Dr. Chen Kang at the College of Pharmacy, Guangzhou University of Chinese Medicine.

Experimental animals

Sprague Dawley (SD) male rats (n = 72) weighing 180-220 g were provided by Guangdong Medical Experimental Animal Center (license number: SYXK AO 2008-0002). Animal welfare and experimental procedures were carried out in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and related ethical regulations of our hospital.

Dose design and grouping

Animals were divided into 9 groups (8 animals each): model, control, and groups A-G. In the group A, piracetam tablets were given at a dose of 360 mg/kg (per 3.6 g/day for an adult). In groups B, C, D, and E, Bajijiasu enteric capsules were administered at doses of 30, 60, 120, and 240 mg/kg, respectively. In group F, Bajijiasu water solution was administered by gavage at a dose of 120 mg/kg. In group G, Bajitian medicinal materials were extracted with water, and the extract was condensed to a dense ointment that was given by gavage on a dose of 1420 mg/kg.

AD remodeling and compound administration

Rats in the model group and all treatment groups received 0.5 mL/100 g (60 mg/kg.d) D-galactose by intraperitoneal (i.p.) injection and 1 mL/100 g (200 mg/kg.d for a rat) aluminum trichloride by gavage. Animals in the control group received physical saline i.p. at a dose of 0.5 mL/100 g and intragastric purified water at a dose of 1 mL/100 g. Both treatments were carried out every morning for 60 consecutive days. The treatment group also received scheduled test drugs once a day for the same duration. Appearances (fur and coloring) and movements were observed, and body weights were recorded daily.

Learning and memory assessment (Morris water maze)

The water maze was performed in a metal circular pool (diameter 120 cm, height 80 cm). A circular Plexiglas platform (diameter 10 cm, height 40 cm) was hidden 2 cm below the water (23 ± 1 °C). The swim speed of rate was in the range of 20-25 cm/s. Experimental rats were initially allowed to swim freely for 2 min to acclimate to the water temperature, environment, and manipulations by staff, such as grasping. Then, they were introduced to the location of the platform for at least 10 s. During the experiment, rats were grasped by their backs facing the pool wall and placed in the water from a fixed entry point for each of the 4 quadrants in the clockwise direction (1→2→3→4). Motion trajectory and escape latency were automatically tracked and recorded by the automated image acquisition system. The experiment lasted for 120 s. If the rats failed to find the platform within 120 s, escape latency was recorded as 120 s. Next, animals were placed on the platform for 10 s to allow them to memorize its location. Rats were trained for 2 consecutive d, followed by 5 d discontinuation. The memory decay experiment was performed on the sixth day.

Activity assays

After collecting the cerebrospinal fluid, the right cerebral hemisphere was harvested, washed in physical saline to remove blood, and weighed after the water was soaked up with filter paper. The sample was diluted to 10% with physical saline in the ice bath, homogenized, and centrifuged at 3500 rpm for 10 min. The supernatant was collected to assay total cholinesterase (TCHE) and SOD activities and MDA content.

Histopathological examination

The left cerebral hemisphere was harvested, fixed with 4% paraformaldehyde solution, embedded in paraffin, and sectioned at a thickness of 5 μm for hematoxylin-eosin (HE) staining. Morphological characteristics were assessed by light microscopy. Two visual fields in each region were selected to count neurons in the basal forebrain, hippocampus, and cerebral cortex.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). SPSS (IBM, USA) was used for statistical analysis. Data were compared among groups with one-factor analysis of variance.

RESULTS

Overall effects

Rats in the control group exhibited normal activity, smooth fur, and sensitive responses. In testing groups, response sensitivity and spontaneous activities were obviously decreased. Food and water intake and body weight also decreased.

Effects on learning and memory

The effects on escape latency during the Morris water maze were studied (Table 1). In the first Morris water maze, the average escape latency of the model group was significantly higher than that of the control group ($P < 0.01$) and the treatment groups with the exception of Group B ($P < 0.05$). In the 24-h Morris water maze, the average escape latency was not significantly different between the model group and the other groups ($P > 0.05$). In the 7-d Morris water maze, the average escape latency of the model group was significantly higher than that of the control group ($P < 0.01$) and Group E ($P<0.05$) but was not statistically different from the other groups ($P > 0.05$).

Effects on TCHE and SOD activities and MDA content

The effects on TCHE and SOD activities and MDA content were investigated (Table 2). TChE activity was not statistically different in the model group compared to the control and treatment groups ($P > 0.05$). However, Groups A and F showed increased activity, and the model group and Groups B-E appeared relatively low, especially Group E that had activity (0.92 ± 0.26) similar to the control group (0.97 ± 0.31). Oral piracetam tablets and intragastric Bajijiasu had no effects on TChE activity in brain tissue, but Bajijiasu enteric capsules reduced TChE activity.

Groups	n	Average escape latency		
		Day 1	Day 2	Day 7
Normal group	8	25.2 ± 4.65	25.6 ± 6.94	16.9 ± 2.15
Model group	7	62.0 ± 30.7$^{\Delta\Delta}$	28.6 ± 7.27	31.7 ± 10.62$^{\Delta\Delta}$
Group A	8	20.3 ± 5.14**	25.7 ± 4.96	23.7 ± 7.51
Group B	8	42.8 ± 25.8	31.9 ± 11.44	29.0 ± 5.62
Group C	8	24.4 ± 7.86**	27.4 ± 9.02	29.6 ± 14.50
Group D	8	27.1 ± 8.09**	21.6 ± 11.34	26.3 ± 13.03
Group E	8	21.3 ± 5.75**	23.0 ± 6.86	19.6 ± 4.43*
Group F	8	30.1 ± 9.87**	30.8 ± 13.5	23.4 ± 9.33
Group G	8	28.1 ± 12.99**	28.9 ± 8.08	32.0 ± 15.80

Table 1. Effects on average escape latency (mean ± SD). $^{\Delta\Delta}$ P < 0.01 *vs*. the control group; * P < 0.05 and ** P < 0.01 *vs*. the model group.

Groups	n	TCHE (u/mL protein)	SOD (u/mL protein)	MDA (u/mL protein)
Control	8	0.97 ± 0.31	270.0 ± 53.0	0.96 ± 0.28
Model	8	1.29 ± 0.89	213.7 ± 43.2$^{\Delta}$	1.44 ± 0.58$^{\Delta}$
Group A	8	1.88 ± 0.85	292.7 ± 75.7*	1.03 ± 0.16
Group B	8	1.09 ± 0.56	219.6 ± 33.9	1.47 ± 0.50
Group C	8	1.05 ± 0.33	301.2 ± 84.9*	1.02 ± 0.33
Group D	8	1.08 ± 0.50	252.0 ± 29.0	1.00 ± 0.14
Group E	8	0.92 ± 0.26	269.1 ± 51.1*	0.94 ± 0.28*
Group F	8	1.84 ± 0.87	228.0 ± 64.3	1.10 ± 0.49
Group G	8	1.24 ± 0.45	240.9 ± 47.5	1.21 ± 0.30

Table 2. TCHE and SOD activities and MDA content in brain tissue (mean ± SD). $^{\Delta}$ P < 0.05 *vs*. the control group; * P <0.05 *vs*. the model group.

As for comparing the normal group and Groups A, C, and E, the model group showed significantly reduced SOD activity (P < 0.05). Both bajijiasu enteric capsules and oral piracetam tablets increased brain tissue SOD activity.

The MDA level was significantly higher in the model group compared to the control group (P < 0.05). Bajijiasu enteric capsules decreased MDA content in rat brain tissue in a dose-dependent manner (P < 0.05), especially in the E group, which showed a level similar to the control group (0.94 ± 0.28 vs. 0.96 ± 0.28).

Effects on neuron number

The effects on neuron number in the basal forebrain, hippocampus, and cerebral cortex of rats were studied (Table 3). The number of neurons in the cerebral cortex in the model group was significantly decreased compared to the control and treatment groups (P < 0.01) with the exception of Group B. The number of hippocampal neurons was significantly decreased in the model group compared to the control group (P < 0.01), and Groups D, F, and G (P < 0.05), but it was not significantly different compared with Groups A, B, C, and E (P > 0.05).

Group	n	Neuron number Cerebral cortex	Hippocampus	Basal forebrain
Control	8	125 ± 11	140 ± 18	140 ± 26
Model	8	72 ± 15$^{\Delta\Delta}$	94 ± 20$^{\Delta\Delta}$	72 ± 21$^{\Delta\Delta}$
Group A	8	112 ± 27**	120 ± 18	117 ± 16*
Group B	8	77 ± 9	95 ± 28	86 ± 19
Group C	8	121 ± 18**	120 ± 26	114 ± 32
Group D	8	125 ± 18**	124 ± 29*	120 ± 7*
Group E	8	125 ± 30**	107 ± 38	111 ± 32
Group F	8	124 ± 15**	125 ± 19*	141 ± 32**
Group G	8	116 ± 24**	124 ± 19*	126 ± 16**

Table 3. Neuron counts in the basal forebrain, hippocampus, and cerebral cortex (mean ± SD). $^{\Delta\Delta}$ P < 0.01 *vs*. the control group; * P < 0.05 and ** P< 0.01 *vs*. the model group; visual field for counting: 0.26 mm^2.

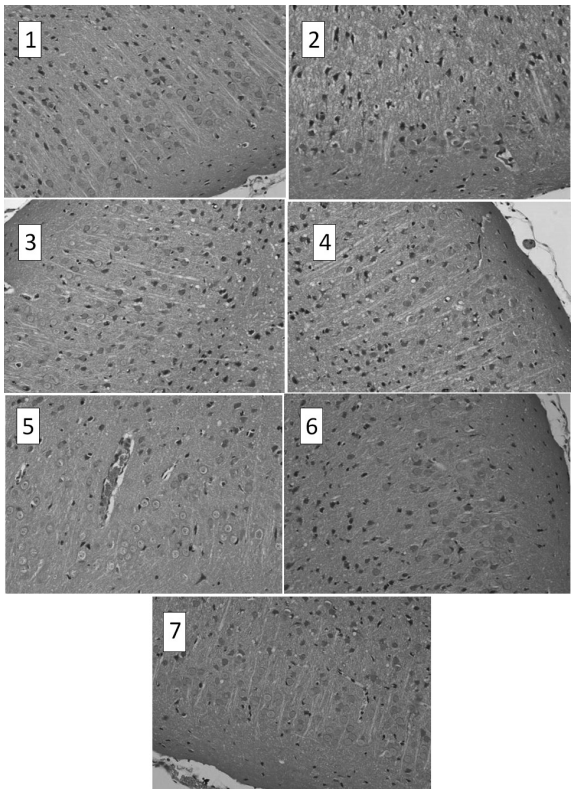

Figure 2. Cerebral cortex neurons, HE ×400. (**1**) Control group; (**2**) Model group: (**3**) Group A; (**4**) Group B; Group D (**5**); (**6**) Group F; (**7**) Group G.

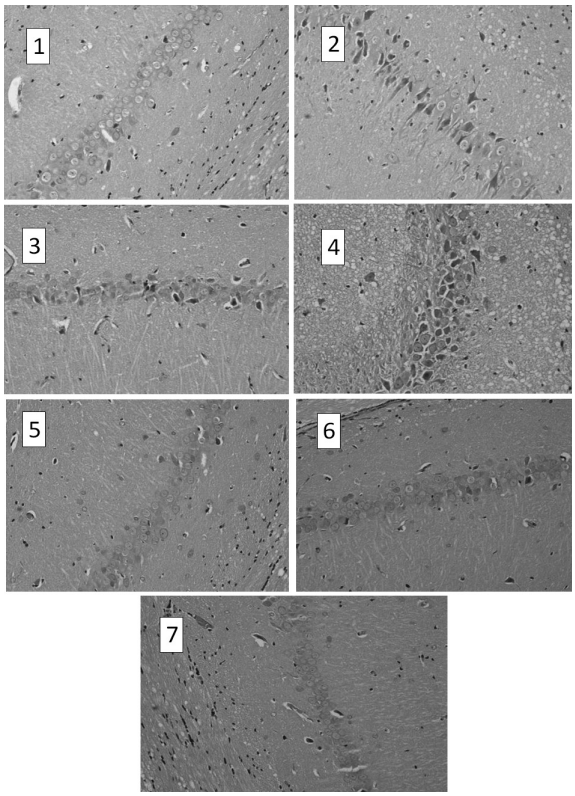

Figure 3. Hippocampal neurons, HE ×400. (**1**) Control group; (**2**) Model group: (**3**) Group A; (**4**) Group B; Group D (**5**); (**6**) Group F; (**7**) Group G.

The number of basal forebrain neurons in the model group was significantly lower than in the control group ($P < 0.01$) and Groups A, D, F and G ($P < 0.05$) but no statistical differences were observed compared with the other treatment groups ($P > 0.05$). Histopathological results are shown in Figs. 2-4.

DISCUSSION

AD is the most prevalent neurodegenerative disease and is strongly correlated with aging. Therefore, aging was simulated by i.p. injections of D-galactose and oral aluminum trichloride to induce neurofibrillary tangles in a rat model of senile dementia.

Bajijiasu doses were determined according to previous reports [7,12]. In this study, groups C, D, and E showed significant improvements in learning and memory ability in a dose-dependent manner. Specifically, the maximal bajijiasu enteric capsule (Group E) effectively attenuated memory decay and inhibited TChE activity to 0.92 ± 0.26, which was significantly lower than the model group (1.29 ± 0.89) and nearly that observed in the control group (0.97 ± 0.31). Although this difference was not statistically signif-

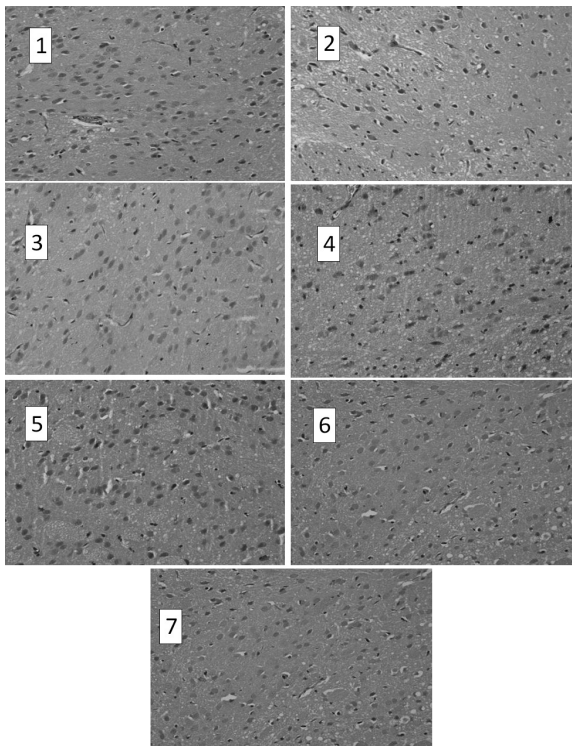

Figure 4. Basal forebrain neurons, HE ×400. (**1**) Control group; (**2**) Model group: (**3**) Group A; (**4**) Group B; Group D (**5**); (**6**) Group F; (**7**) Group G.

icant, it suggests that AChE was inhibited by raw bajijiasu. AChE is a glucoprotein, and previous studies have demonstrated a low binding capability of bajijiasu and serum proteins in rats [13]. In the current study, TChE activity was decreased in the treatment groups compared to the model group, but the differences were not statistically significant. More studies are needed to investigate the relationship between the obtained result and a low binding capability.

Bajijiasu treatment had several other important effects, regardless of administration route. Moreover, it also increased SOD activity, decreased MDA content, and attenuated neuronal loss in the basal forebrain, hippocampus, and cerebral cortex. While we did not observe any statistical differences in brain tissue MDA content or cerebral cortex and hippocampus neuron number between Groups D (enteric capsule) and F (gavage) that provided the same dose, we did observed that compared with Group F, Group D significantly reduced the activities of TChE (1.08 ± 0.50 *vs.* 1.84 ± 0.87) and SOD (252.0 ± 29.0 *vs.* 228.0 ± 64.3). These results suggest that administration route was important, and might be due to the stability of bajijiasu in the intestine. This observation indicates that the administration route should be considered in the subsequent studies to ensure bajijiasu efficacy.

The neuron counts in the different brain regions suggest comparable efficacy among enteric capsules, gastric perfusion, and extract administration, illustrating that bajijiasu nourishes the kidney and benefits the brain. These results support the theory that "the brain is correlated to the kidney" [14,15] and suggest that bajijiasu has anti-AD effects and should be furthered developed as a possible AD therapy.

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