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The Effects of Martebe Juice Polyphenol on the Memory Acquisition Impairment of Mice Induced by Scopolamine

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Abstract: As for the anile model mice fed with scopolamine, Martebe juice polyphenol can improve their ability of learning and memory, strengthen the function of the oxidation-resistance system of mice, increase the mass index of spleen and thymus, strengthen immunologic function and restrain the necrosis of hippocampus cells of the anile mice. It has prominent function of delaying senescence. As for the memory acquisition impairment of mice induced by scopolamine, Martebe juice polyphenol has certain function of improvement.

Keywords Martebe Juice Polyphenol, Memory Acquisition Impairment, Mice Induced, Scopolamine

INTRODUCTION

Martebe Juice is a kind of non-fermentative juice. Its main active ingredient is Martebe juice polyphenol (GTP). GTP is generic term of the phenols in Martebe juice. The content of catechin is most among the phenols[Jang, *et. al.*, 2014]. In the research during the latest years, GTP has many biological activities of oxidation resistance, relieving the damage of free radicals, anti-aging, atherosclerosis prevention, anti-cancer, antibiosis and anti-inflammation.

Senescence is a natural phenomenon that is ubiquitous in living nature[Sacco, *et. al.*, 2015]. After body becomes mature, it will become senescent with age increase and finally die. The main performances can be: organs and tissues age; metabolism velocity slows down; various physiological functions decline. In order to better research the mechanism of senescence, many animal models are applied in experiments [Walker, *et. al.*, 2013]. At present, commonly-used senescence models include senescence model of ozone injury, senescence model of thymus extirpation, speedy aging model (SAM) of mice, D-galactose sub-acute senescence model and natural senescence model. D-galactose sub-acute senescence model is built through injecting D-galactose into the subcutaneous tissue of the back of mice [Jain, *et. al.*, 2015]. The model mice show the sign of senescence which is similar to that of the mice with natural senescence. D-galactose senescence model was raised by Chinese scholar in the middle term of the 1980s. This model is simple and stable and can be built in short time (1.5-2 months) with low cost. At present, it is the most popular senescence model in our country. Based on the theory, this experiment uses D-galactose sub-acute senescence model to detect their corresponding ethology indexes and biochemical indexes, observe the anti-aging function of GTP on the D-galactose sub-acute senescence model mice and explore its mechanism of action [Pervin, *et. al.*, 2017]. Up to now, domestic experimental researchers have found that GTP can well improve the learning and memory impairment induced by D-galactose to mice. Through measuring the activity of superoxide dismutase (SOD) in serum, it is speculated that the learning and memory improvement function of juice polyphenol may be related to the activity of SOD [Wang, *et. al.*, 2016]. However, the effects of GTP on hippocampus cells have not been reported. Through reading relevant literatures, the author knows that hippocampus nerve cells are closely related to senescence. Hippocampus is a key part of brain in senescence. In the process of development and senescence, hippocampus has remarkable change of morphological structure and cells' structure. Therefore, the research on the change of the morphological structure of hippocampus tissue of the mice in senescence contributes to further revealing the anti-aging function and mechanism of GTP.

MATERIALS AND METHODS

Experimental animals

Healthy male 4-month-old Kunming mice are chosen in the experiment (offered by the Experimental Animal Center of Shandong Lukang Drugs Co., Ltd.; the number of qualification certificate: SCXK (Lu) 2008-0002). Their weight is 20±2.0g and they are raised in different cages with free eating and drinking. Fodders are the standard fodders for experimental animals supplied by the Experimental Animal Center of Shandong Lukang

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Drugs Co., Ltd. The temperature in lab is controlled at 25±2℃. The lab is cleaned every day with natural illumination and ventilation. After the animals adapt for the environment for 7 days, ethology screening experiment will be operated.

Drugs and reagents

Martebe juice polyphenol (content 98%): Zhengzhou Linuo Biotechnology Co., Ltd., batch number: CDF20120302; piracetam: Jilin Shuangyao Pharmaceutical Group Co., Ltd., batch number: 20111001; scopolamine hydrobromide: Shanghai Harvest Pharmaceutical Co., Ltd., batch number: 110402; normal saline: Shijiazhuang No.4 Pharmaceutical Co., Ltd., batch number: 110824405.

Main instruments and equipment

Second chronograph: Shanghai Second Chronograph Factory; program-control water maze: Institute of Pharmacology of Chinese Academy of Medical Sciences, type SMG-2.

Experimental method

Animals grouping and modeling

Kunming mice with weight 22±2g are firstly raised in lab for 7 days to adapt to the environment and then preliminarily selected. The mice are put in jumping stand experiment with voltage 24V. The excessively sensitive mice (instant electric seizure) and the excessively insensitive mice (without eluding electric shock) are eliminated. 60 qualified mice are finally selected and randomly divided into 6 groups: normal comparison group, scopolamine model group, the group with low dosage of Martebe juice polyphenol (100 mg/kg), the group with middle dosage of Martebe juice polyphenol (250 mg/kg), the group with high dosage of Martebe juice polyphenol (625 mg/kg) [4, 5] and piracetam group. Gavage is used to the groups according to their different dosage. Normal saline with the same volume is given to the mice of normal comparison group and model groups. Volume of drug is 0.1ml/10g for 1 time every day. Gavage lasts for 14 days. From the 15th day, gavage is firstly operated to the mice and the method is same as before. 2 hours later, the mice in normal comparison group are injected with normal saline 2mg/kg; the mice of other groups are injected with scopolamine in the same volume. 30 minutes later, jumping stand experiment is operated. 24 hours later, jumping stand experiment is operated again to test the memory maintenance ability of the mice. From the 17th day, water maze test is operated for 4 days. During the first 3 days, gavage is operated to all mice every day and the method is same as before. 30 minutes later, the mice in normal comparison group are injected with normal saline 2mg/kg; the mice of other groups are injected with scopolamine in the same volume. 30 minutes later, water maze test is operated. On the 4th day, gavage is operated as before. 2 hours later, water maze test is then operated.

Water maze test:

The device of water maze is illustrated in figure 1. Water temperature is (25.0±1.0) ℃ and water depth is 10cm.

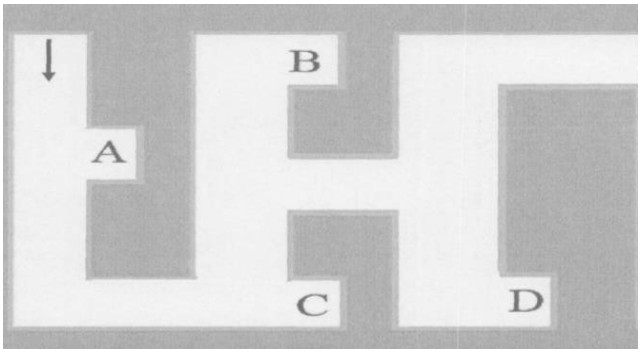

Figure 1 The device of water maze

In the passageway-type water maze, A, B, C and D are four blind ends; the arrow is starting point; the step is terminal platform.

Passageway-type water maze is used in the experiment. The water maze is made of double-layer organic glass in dark color with size 80cm×50cm×20cm. Starting point, terminal platform and 4 blind ends are set in it. Animal grouping and drug gavage are same as those in jumping stand experiment. From the 17th day to the 19th day, the mice are trained. They are tested on the 20th day and the data is regarded as memory score. Before each training, put mice in the water beside the terminal platform and make them climb up the step for 2 times. 2 blind ends are set on the 17th day and mice are put at the starting point to make them swim to the terminal point for 2 times as training. 3 blind ends are set on the 18th day; 4 blind ends are set on the 19th day and the 20th day. The training method is same as that used on the 17th day. When the experiment begins, put mice at the starting point facing the wall of the maze; then record the duration of the swim from the starting point to the terminal point (latent period) and record the times of entering blind end of the maze.

If any mouse cannot swim out of the maze within 3 minutes, 3 minutes will be recorded as duration. If any mouse cannot reach the step within stipulated

time, it will be guided to reach the step and stay there for 20 seconds. Statistically process the data to evaluate the effects of Martebe juice polyphenol in the water maze test on the memory acquisition impairment of mice induced by scopolamine.

RESULTS AND DISCUSSION

In the water maze test, comparing with the mice in normal comparison group, the mice in model group have much more times of entering blind ends (namely the times of error) ($P<0.01$); and the duration of swimming to the terminal point (latent period) is much longer ($P<0.01$). Comparing with the mice in model group, the mice in the groups of low-dosage, middle-dosage and high-dosage Martebe juice polyphenol and piracetam group have less times of error ($P<0.05$ or $P<0.01$) and shorter latent period ($P<0.05$ or $P<0.01$). In addition, the data of the groups of Martebe juice polyphenol in different dosages has no difference from that of piracetam group. The results indicate that Martebe juice polyphenol can improve the memory acquisition impairment of mice induced by scopolamine. Test results are shown in table 1.

Tab.1 Effects of GTP on acquired learning and memory disorder in mice induced by scopolamine in water maze test (±s)

Group	Quantity	Dosage (mg/kg)	Latent period (s)	Times of error
Normal comparison group	10		82.3±14.4	4.8±1.4
Model group	10		159.0±26.2$^{\#}$	9.4±2.7$^{\#\#}$
Martebe juice polyphenol group (low dosage)	10	100	112.1±11.9*	6.2±1.4*
Martebe juice polyphenol group (middle dosage)	10	250	106.4±18.0*	6.2±1.9*
Martebe juice polyphenol group (high dosage)	10	625	83.2±18.2**	5.6±1.4*
Piracetam group	10	600	100.2±22.9**	4.8±1.6**

Comparing with normal comparison group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; comparing with model group, $*P<0.05$, $**P<0.01$

With the increase of age, the cells, tissues and organs of animal gradually degenerate and become senescent. For example, organs atrophy; weight is lost; memory retrogresses. The most noteworthy senescence is the retrogression of memory. Memory impairment is the common symptom of senescence and senile diseases.

The effects of drugs on learning memory are mainly observed by the method of ethology. Behavior experiments are widely applied in the research on learning memory. In memory research, one of the most important animal models is to restrain the imitative learning habit of live animals. Passive avoidance means that animals eliminate certain behavior and avoid certain annoying matter through being trained. Inhibitory avoidance is often applied in the experimental research on anti-aging. Jumping stand experiment is behavioral experiment which can objectively reflect the memory acquisition and consolidation after suffering electric shock.

Spatial differentiating learning is a kind of associative learning. It needs animals to regard objects around spatial position as reference substances, discern them again and again and relate the reference substances to spatial position in their brain. With the help of reference substances, the ability of recognizing spatial positions can be got. Water maze test is a behavioral model related to spatial learning memory.

CONCLUSION

In this research, we operate two different ethology tests to reflect the change of learning memory ability of mice in their senescence process. Jumping stand experiment reflects the passive avoidance ability of mice; water maze test reflects spatial learning

memory ability of mice. The two ethology indexes can objectively reflect the learning memory of mice. Experimental result indicates that GTP can well improve the sub-acute senescence induced by D-galactose and improve the memory impairment induced by scopolamine. GTP in different dosages can prominently prolong the latent period of jumping down stand, shorten the latent period of seeking the stand in water maze, decrease the times of error, effectively improve the learning memory ability induced by D-galactose and improve the memory impairment induced by scopolamine.

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