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Preparation of total saponins from *Panax japonicus* and their protective effects on learning and memory ability of aging mice

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Abstract

This study aimed to prepare the total saponins from *Panax japonicus* (TSPJ), and investigate their protective effects on learning and memory ability of aging mice induced by *D*-galactose. The TSPJ product with content of 81.03% was obtained. Fifty mice were randomly divided into control, model, and low-, middle- and high-dose TSPJ groups. The *D*-galactose-induced aging model was established in model, and low-, middle- and high-dose TSPJ groups. Then, the low-, middle- and high-dose TSPJ groups were treated with 20, 40 and 80 mg/kg TSPJ by gavage, respectively, for 8 weeks. At the end of treatment, compared with model group, in 40 and 80 mg/kg TSPJ groups the brain aging symptoms were obviously moderated, the step-down latency was increased, the frequency of mistake was decreased, the brain index was increased, the brain tissue SOD and GSH-Px levels were increased, the brain tissue MDA level was decreased, and the brain tissue Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities were increased. In conclusion, TSPJ can reduce the brain tissue oxidative stress injury and protect the cell membrane transport capacity, thus improving the learning and memory ability in aging mice.

Keywords: total saponins; *Panax japonicus*; memory; aging; mice.

Practical Application: This study has provided a basis for preparation of total saponins from *Panax japonicus* and their medicinal application.

1 Introduction

Aging is a time-dependent decline of the functions of various tissues and organs in the body, and it is the final stage of any life after maturity. Aging is mainly manifested by the gradual weakening or even loss of the body's ability to adapt to the environment. The brain is the main regulator of life function. The body aging can cause the brain aging-related neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease and so on. These diseases lead to the physical function and behavioral ability disorders, and affect the elderly in physiology, psychology, intelligence and other aspects (Bonaconsa et al., 2013; Ljubisavljevic et al., 2013). Despite the deepened study on mechanism of aging (Sohal et al., 2002; Bishop et al., 2010), the research progress of anti-aging methods and drugs is relatively backward, and it is difficult to find a drug that can take into account many aspects of aging. Therefore, it is of positive scientific significance to seek an active and effective drug to delay the aging, prevent the geriatric diseases, shorten the sick and disabled period, and improve the life quality of the elderly. *Panax japonicus* is one of the unique medicinal plants growing in China. Modern pharmacological studies have shown that *Panax japonicus* contains many active ingredients which have the antioxidant, anti-inflammatory, immune-regulating, myocardial ischemia protecting, and other pharmacological effects (He et al., 2012; Yang et al., 2014; Jie et al., 2015; Deng et al., 2017). Total saponins are the main active component of *Panax japonicus*. In this study, the total saponins from *Panax japonicus* (TSPJ) were prepared, and their protective effects on learning and memory ability of aging mice induced by D-galactose were investigated. The objective was to provide an experimental basis for the clinical application of TSPJ to aging-related neurodegenerative diseases.

2 Materials and methods

2.1 Preparation of TSPJ

Dry rhizomes of *Panax japonicus* were smashed. The powder was added to the extraction kettle, followed by adding 10 times (volume to mass) of 60% ethanol solution. After soaking for 2 h, the heat reflux extraction was performed for three times, 2 h each time. The filtrates of three times were combined, and concentrated by rotary evaporation. The residues were dissolved with hot water, followed by extraction with n-butanol for three times. The extraction solutions of three times were combined.

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After removing the solvent by rotary evaporation, the residues were dissolved with 85% ethanol. Then, 5 times (volume to that of 85% ethanol) of acetone was slowly added to precipitate. Finally, the precipitate was obtained. It was confirmed as the TSPJ product by HPLC, and the content of total saponins was 81.03%.

2.2 Grouping of mice and establishment of aging model

Fifty Kunming mice (half male and half female; 20-22 g) were adaptively fed for one week. Then, the mice were randomly divided into control, model, and low-, middle- and high-dose TSPJ groups, with 10 mice in each group. The subacute aging model was established in latter four groups. A 10 g *D*-galactose was dissolved in 500 mL normal saline, and then was given to the mice by intraperitoneal injection, with dose of 200 mg/kg. The mice in control group were given with the same volume of normal saline by intraperitoneal injection. The injection was performed once every day, for 8 weeks. The body weight of mice was weighed once a day, and the dose of *D*-galactose was adjusted according to the body weight.

2.3 Treatment of mice

From the ninth week, the mice in low-, middle- and high-dose TSPJ groups were given 20, 40 and 80 mg/kg TSPJ by gavage, respectively. The mice in control and model groups were given the same volume of normal saline. The treatment was performed once every day, for 8 weeks. During the treatment, the general state of mice was observed.

2.4 Step-down test

Two hours after the last administration, the learning and memory ability of mice was detected by step-down test. The mice were placed in step-down test apparatus to adapt to the environment for 3 min. Then, the alternating current (36 V, 2 mA) was powered on. The normal reaction of mice was to jump to the safe platform after being electrically shocked, followed by jumping to the electrified copper grid. The mice jumped for several times between safe platform and electrified copper grid. The training was performed for 5 min. After 24 h, the mice were placed on the safe platform, the time before first jumping to the electrified copper grid within 5 min was recorded as the step-down latency, and the number of electrically shocks within 5 min was recorded as the frequency of mistake.

2.5 Detection of brain index

After step-down test, the mice were killed by decapitation. The brains were taken and weighed. The brain index was calculated according to the formula: brain index (mg/g) = brain mass (mg) / body mass (g).

2.6 Detection of biochemical indexes in brain tissues

The brain tissues were taken, and were homogenized with pre-cooled normal saline. After centrifuging at 3000 r/min for 10 min, the supernatant was obtained. The oxidative stress indexes including superoxide dismutase (SOD) activity and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content and cell membrane transport capacity indexes including Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities were detected using the corresponding kits.

2.7 Statistical analysis

All statistical analyses were performed using SPSS software (ver. 22.0). One-way analysis of variance (ANOVA) followed by the least significant difference method (LSD) was adopted for comparison of data among five groups. Data are expressed as mean ± standard deviation. Statistical significance was set at $P < 0.05$.

3 Results

3.1 General state of mice

During the experiment period, the mice in control group had normal diet, flexible action, god-catching eyes, quick response and bright hair. No mouse died. In model group, the mice in gradually appeared the phenomena of weakness of limbs, lack of vision, slow action, slow response, yellow hair color, soft skin, and red stool. Two mice died. In three TSPJ groups, before treatment the mice had the symptoms similar with those in model group. However, after treatment, the symptoms in three TSPJ groups were obviously moderated compared with those in model group, especially for the high-dose TSPJ group. No mouse died in three TSPJ groups.

3.2 Step-down test results

At the end of treatment, the step-down test showed that, compared with control groups, in model and low-, middle- and high-dose TSPJ groups the step-down latency was significantly decreased, respectively ($P < 0.05$), and the frequency of mistake was significantly increased, respectively ($P < 0.05$). Compared with model group, the step-down latency in middle- and high-dose TSPJ groups was significantly increased, respectively ($P < 0.05$), and the frequency of mistake in low-, middle- and high-dose TSPJ groups was significantly decreased, respectively ($P < 0.05$) (Table 1).

3.3 Brain index

Figure 1 showed that, at the end of treatment, when comparing with control group, the brain index in model and three TSPJ

Table 1. Comparison of step-down latency and frequency of mistake among five groups.

Group	n	Step-down latency (s)	Frequency of mistake (times/5 min)
Control	10	176.89 ± 29.05	1.80 ± 0.13
Model	8	44.34 ± 7.72[a]	9.91 ± 2.04[a]
Low-dose TSPJ	10	56.27 ± 5.38[a]	7.52 ± 1.45[ab]
Middle-dose TSPJ	10	62.08 ± 9.49[ab]	6.18 ± 0.84[abc]
High-dose TSPJ	10	84.20 ± 12.53[abcd]	4.43 ± 0.39[abcd]
F		113.762	66.304
P		0.000	0.000

[a]$P < 0.05$ *vs.* control group; [b]$P < 0.05$ *vs.* model group; [c]$P < 0.05$ *vs.* low-dose TSPJ group; [d]$P < 0.05$ *vs.* middle-dose TSPJ group; TSPJ = total saponins from *Panax japonicus*.

Table 2. Comparison of brain tissue SOD, GSH-Px and MDA levels among five groups.

Group	n	SOD (U/mg)	GSH-Px (U/mg)	MDA (nmol/mg)
Control	10	145.05 ± 27.05	75.04 ± 11.09	5.62 ± 1.06
Model	8	82.16 ± 15.37[a]	36.20 ± 6.80[a]	10.12 ± 1.67[a]
Low-dose TSPJ	10	94.34 ± 12.20[a]	42.38 ± 7.52[a]	9.01 ± 1.32[a]
Middle-dose TSPJ	10	104.29 ± 15.63[ab]	58.18 ± 9.71[abc]	8.12 ± 1.52[ab]
High-dose TSPJ	10	124.61 ± 17.18[abcd]	61.29 ± 8.29[abc]	7.04 ± 1.30[abc]
F		17.371	28.167	14.754
P		0.000	0.000	0.000

[a]P < 0.05 *vs.* control group; [b]P < 0.05 *vs.* model group; [c]P < 0.05 *vs.* low-dose TSPJ group; [d]P < 0.05 *vs.* middle-dose TSPJ group; TSPJ = total saponins from *Panax japonicus*; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

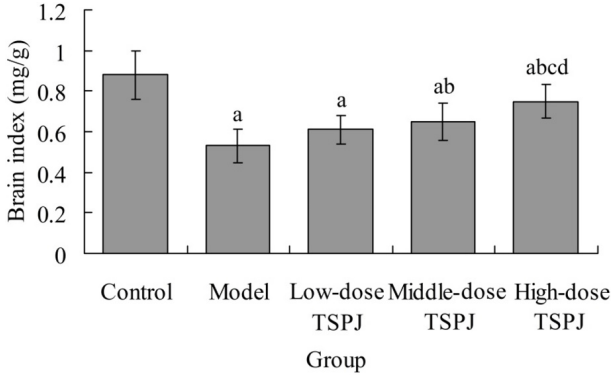

Figure 1. Comparison of brain index among five groups (F = 21.001, P = 0.000). [a]P < 0.05 *vs.* control group; [b]P < 0.05 *vs.* model group; [c]P < 0.05 *vs.* low-dose TSPJ group; [d]P < 0.05 *vs.* middle-dose TSPJ group; TSPJ = total saponins from *Panax japonicus*.

Table 3. Comparison of Na^+-K^+-ATPase, Ca^{2+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities among five groups.

Group	n	Na^+-K^+-ATPase [μmol/(mg·h)]	Ca^{2+}-Mg^{2+}-ATPase [μmol/(mg·h)]
Control	10	8.19 ± 0.23	6.07 ± 1.02
Model	8	4.38 ± 0.37[a]	2.76 ± 0.34[a]
Low-dose TSPJ	10	4.94 ± 0.56[a]	3.14 ± 0.41[a]
Middle-dose TSPJ	10	6.50 ± 1.04[abc]	4.17 ± 0.52[abc]
High-dose TSPJ	10	7.29 ± 1.06[abcd]	4.59 ± 0.72[abc]
F		41.851	36.810
P		0.000	0.000

[a]P < 0.05 *vs.* control group; [b]P < 0.05 *vs.* model group; [c]P < 0.05 *vs.* low-dose TSPJ group; [d]P < 0.05 *vs.* middle-dose TSPJ group; TSPJ = total saponins from *Panax japonicus*.

groups was obviously decreased, respectively (P < 0.05). When comparing with model group, in middle- and high-dose TSPJ groups the brain index was obviously increased, respectively (P < 0.05).

3.4 Brain tissue SOD, GSH-Px and MDA levels

At the end of treatment, compared with control groups, in model and low-, middle- and high-dose TSPJ groups the brain tissue SOD and GSH-Px levels were significantly decreased, respectively (P < 0.05), and the brain tissue MDA level was significantly increased, respectively (P < 0.05). Compared with model group, in middle- and high-dose TSPJ groups the SOD and GSH-Px levels were significantly increased, respectively (P < 0.05), and the MDA level was significantly decreased, respectively (P < 0.05) (Table 2).

3.5 Brain tissue Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities

As shown in Table 3, at the end of treatment, in model and low-, middle- and high-dose TSPJ groups the brain tissue Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities were significantly lower than those in model group, respectively (P < 0.05). Compared with model group, the Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in middle- and high-dose TSPJ groups were significantly increased, respectively (P < 0.05).

4 Discussion

Studies have found that many plant extracts have good pharmacological effects (Lee et al., 2020; Wu et al., 2020; Santos et al., 2021). As TSPJ are the main active ingredients of *Panax japonicus*, a common medicinal plant growing in China, this study prepared the TSPJ, and investigated their pharmacological effects. There are many animal models to study the aging and dementia. The mouse aging model induced by injecting *D*-galactose is a common subacute aging model established according to the metabolism theory of aging. It has the advantages of short modeling time, simple operation and good reproducibility, and has become a recognized method to simulate animal aging model. In this study, the aging model of mice induced by *D*-galactose was established, and the protective effects of TSPJ on learning and memory ability of aging mice were investigated. Results showed that, compared with model group, in TSPJ groups the brain aging symptoms were obviously moderated, the step-down latency was increased, the frequency of mistake was decreased, and the brain index was increased. This indicates that, TSPJ can reduce brain injury and improve the learning and memory ability in aging mice induced by *D*-galactose.

In recent years, great progress has been made in the study of aging. Researchers have proposed a number of theories related to aging. Among them, the theory that the oxidative stress is closely related to the mechanism of brain aging is generally accepted (Finkel & Holbrook, 2000). Zeng et al. (2014) have shown that

the free radicals are increased significantly in the brain tissue of naturally aging and *D*-galactose induced aging rats. de la Torre et al. (1998) have found that inhibiting free radical injury can effectively reverse the neuron injury and prevent brain aging. In addition, SOD and GSH-Px can effectively eliminate oxygen free radicals in the body, so as to protect the body from the oxidative stress injury (Devi et al., 2000). MDA is an aldehyde produced by free radical-induced lipid peroxidation, and its content can reflect the degree of lipid peroxidation (Tsikas, 2017). In this study, at the end of treatment, compared with model group, in TSPJ groups the brain tissue SOD and GSH-Px levels were increased, and the MDA level was decreased. It is suggested that TSPJ can effectively reduce the oxidative stress, so as to play a protective role in the brain tissue of aging mice.

In the process of aging, the imbalance of Ca^{2+} often leads to the overload of intracellular Ca^{2+}, which may be one of the reasons for the degeneration of nervous system (Calvo-Rodríguez et al., 2016). It is found that, the intracellular Ca^{2+} overload is closely related to aging diseases, and the activities of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase are the basis of Ca^{2+} distribution, which are used as the new indicators of aging (Torlińska & Grochowalska, 2004). The decrease of Na^{+}-K^{+}-ATPase activity can lead to the disturbance of intracellular ATP production and sodium transport, thus affecting the overall function of cells (Fisher & Margulies, 2002). The decrease of Ca^{2+}-Mg^{2+}-ATPase activity can cause the increase of intracellular Ca^{2+} concentration, leading to the instability of cell skeleton and membrane structure and increase of membrane permeability (Fu et al., 1998). In our study, compared with model group, the brain tissue Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in TSPJ groups were increased. This suggests that, TSPJ can protect the cell membrane transport capacity, thus alleviating the brain injury and improving the learning and memory ability in aging mice.

5 Conclusion

In conclusion, TSPJ can reduce the brain tissue oxidative stress injury and protect the cell membrane transport capacity, thus improving the learning and memory ability in aging mice. This study can provide an experimental basis for the medicinal application of TSPJ to aging-related neurodegenerative diseases. The other action mechanisms of TSPJ still need to be further clarified.

Conflict of interest

The authors declare that there is no conflict of interest.

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