Antioxidant effect of peony seed oil on aging mice

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Abstract D-galactose was injected into mice of ages 4–5 months, and peony seed oil was administered using an oral gavage to assess its possible anti-aging functions. The content of malondialdehyde (MDA) and the activities of monoamine oxidase (MAO), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in the brain and liver of these mice were determined using biochemical kits. The significance of the differences in the content of the components associated with aging and anti-aging among each group was analyzed statistically. The MDA content and activities of MAO in the brain and liver of mice in the peony seed oil group were significantly lower than those in the aging group. The activities of GSH-Px, Cu/Zn-SOD, and Mn-SOD in the brain and liver of mice in the peony seed oil group were very significantly higher than those in the aging group. Peony seed oil was determined to have an obvious anti-aging function.

Keywords Peony seed oil · Aging · Anti-aging · Superoxide dismutase

Introduction

Peony (*Paeonia suffruticosa* Andr.) is medicinal and ornamental plant. In China, it has been used for over 2000 years now. The medicinal part of peony is its dry root bark (Dan Pi), which promotes blood circulation and removes blood stasis, which is commonly performed when treating cardiovascular diseases, inflammations, allergies, and so on [1–4]. Seeds of peony are rich in oil [5, 6].

Peony seed oil is one of the functional foods that have been newly approved by the Chinese government. This oil's content of polyunsaturated fatty acids exceeds 90%, and the content of linolenic acid is up to 40% [7–9]. Studies have shown that peony seed contains peony phenol; peony sterol; apigenin; trans-resveratrol; β -sitosterol; stigmasterol; suffruticosol A, B, C, and D; 8-debenzoyl-paeonidanin; albiflorinR1; peoniflorin; nicotinic acid; vitamins E; caffeic acid; oleanolic acid; and so on [10–13].

Recent studies have shown that peony seed oil is non-toxic and safe and has a very high nutritional value and many health benefits, including an antioxidant function, stimulation of blood circulation, lowering effect on blood glucose, prevention and treatment of diabetes mellitus, hepatoprotective effect, hypolipidemic effect, anti-UV and sunscreen effect, anti-tumor activity, antianaphylaxis, anti-inflammation, cardiovascular protection, anti-atherosclerosis, neuroprotective effect, reducing skin pigmentation, strengthening nonspecific immunity, relieving climacteric syndrome and rheumatoid arthritis, and so on [14–18]. Thus, peony seed oil is known as one of the “blood nutrients” and “plant brain gold” and has been processed

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into functional foods [19]. However, there is no systematic and in-depth research on the anti-aging effects of peony seed oil on aging animals.

During aging, the content of malondialdehyde (MDA) and the activity of monoamine oxidase (MAO) increase, whereas the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) decrease. MDA can promote the formation of lipofuscin and the age pigment and accelerate aging. MAO can increase the breakdown of monoamine neurotransmitters, affect brain health, and promote aging. SOD and GSH-Px can accelerate the removal of free radicals and peroxides and delay senility [20–22]. Therefore, the content of MDA and the activities of MAO, SOD, and GSH-Px are important parameters for assessing the severity of body aging.

The aging animal model induced by D-galactose was integrated with the aging animal model design based on the principle of metabolic disturbance in the aging process [23–25]. In the animal body, D-galactose was reduced to galactitol by aldose reductase. The galactitol cannot be metabolized and accumulates in the cell, which can cause metabolic disturbance, Oxidative stress-free radical damage, the activity of antioxidant enzymes (SOD and GSH-Px) decreased, while the activity of MAO and the MDA content increased. On the other hand, D-galactose was transformed to advanced glycation end-products (AGEs) via nonenzymatic glycation. AGEs binding to the AGE receptor activated the signal pathway associated with AGEs, which can promote lipid peroxidation, strengthen the roles of the above-mentioned adverse effects on the body, and accelerate the aging of the organism. Each organ of the aging animal model exhibits different degrees of senility symptoms. Therefore, this aging model was an ideal experimental tool for studying the anti-aging effect and has been widely used to study the anti-aging action of drugs and functional foods [26–28].

To investigate the health effects of peony seed oil on aging mice, we administered the oil using an oral gavage to aging model mice induced by D-galactose and determined the components associated with aging and anti-aging.

Materials and methods

Materials

Fengdan peony seed oils were purchased from Shandong Heze Ruipu Peony Industry Technology Development Corporation, China.

Determination of peony seed oil dosage

Dong et al. [29] and Liu et al. [30] found that peony seed oil at a dose of 6 mL/kg body weight (BW)/day, significantly reduced the blood lipid content and had a significant antioxidant effect on hyperlipidemia mice. Based on these studies, we determined that peony seed oil dosage was 6 mL/kg BW/day.

Construction of the mouse model of the aging and anti-aging experiment

Following a 7-day acclimation period, 30 healthy male mice (Tianqing Biological Technology Co., Ltd, Changsha, China), 4–5 months of age and with BWs of 30 ± 5 g, were randomly assigned to 1 of three groups [1 peony seed oil group (PSOG), 1 aging model group (AMG), and 1 control group (CG)] comprising ten mice each. The mice were allowed free access to food and water. The experimental design was in accordance with the guidelines for animal experimentation. This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the Changsha, China. (Changsha Tianqin Biotechnology Co., Ltd, approval number: Scxk (xiang) 2014-0011).

The basic feed (Tianqing Biological Technology, Changsha, China) constituted flour (20%), rice flour (10%), corn flour (20%), wheat bran (25%), bean (20%), bone meal (2%), and fish meal (2%).

The mice in the PSOG and in the AMG were subcutaneously injected with D-galactose (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) at a dose of 125 mg/kg BW/day [23–25] once daily for 6 weeks, whereas those in the CG were treated with the same volume (0.2 mL) of physiological saline. After injecting D-galactose, the mice in the PSOG were administered peony seed oil at a dose of 6 mL/kg BW/day using an oral gavage, whereas the mice in the AMG and the CG were administered the same volume of distilled water. Clinical observations were made following this treatment. The general conditions (appearance, behavior, dejecta, and temperament) and food consumption of all animals were recorded every day. The BWs of the mice were measured at commencement of the experiments and at the end of every other week.

Determination and analysis of components associated with aging and anti-aging

At the end of the sixth week, the animals were euthanized to undergo a gross pathological examination. The brain and liver were then removed from each cadaver. The brain and liver were homogenized (physiological saline = 1:9 for both) on ice according to the procedure described by Jin

et al. [31], and the supernatant liquid of the homogenate was removed by centrifuging ($1048\times g$) for 10 min for biochemical analysis (AqDL-5-8 centrifuge, Shanghai Xucheng Instrument Company, Shanghai, China). The content of MDA, the content of total protein (TP), and the activities of total-SOD (T-SOD), copper/zinc-SOD (Cu/Zn-SOD), GSH-Px, and MAO were determined using the appropriate biochemical analysis kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and the UV752N ultraviolet–visible spectrophotometer (Inesa Analytical Instrument, Shanghai, China) in accordance with the respective kit instructions. MAO activity was determined using a monoamine oxidase assay kit (Colorimetric method). MDA content was determined using a malondialdehyde assay kit (thiobarbituric acid test method). GSH-Px activity was determined using a glutathione peroxidase assay kit (colorimetric method). Content of TP was determined using a total protein quantitative assay kit. Activities of T-SOD and copper/zinc-SOD (Cu/Zn-SOD) were determined using a superoxide dismutase typed assay kit (hydroxylamine method). The activity of manganese-SOD (Mn-SOD) was obtained by subtracting the activity of Cu/Zn-SOD from that of T-SOD.

Statistical analysis

All data are expressed as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA and the Duncan test to explore the differences between groups (SPSS 16.0). Values having $p < 0.05$ were considered statistically significant.

Results and discussion

Appearance, behavior, dejecta, temperament, BW, and food consumption of mice

No mice died during the experiment. At the end of the testing period, the mice in the AMG exhibited obvious senility symptoms, such as reduced activity, sparse, dull yellow fur, irritable temperament, and thin dejecta. The appearance, behavior, dejecta, and temperament of mice in the PSOG were similar to those of the CG: they were in good condition; their temperament was mild; they displayed agility and nimbleness in their movements; their dejecta was normal; and their fur was thick and shiny (Table 1).

A summary of the total BWs are presented in Table 2A. The results show that the BW gain of the mice in the AMG was significantly lower than that of the mice in the CG ($p < 0.05$) at the end of the experiment. By contrast, the BW gain of the mice in the PSOG was significantly higher

than that of the mice in the CG ($p < 0.05$) and the AMG ($p < 0.01$).

The food consumption values are presented in Table 2B. The results show that the total food consumption values for mice in the AMG were significantly lower than those of mice in the CG ($p < 0.05$). The total food consumption values for mice in the PSOG were lower than those of the mice in the CG and higher than those of the mice in the AMG, but no significant difference was detected ($p > 0.05$).

Effect of peony seed oil on MDA content and MAO and GSH-Px activities

The MDA content and the activities of MAO and GSH-Px in the brain and liver of the mice in each group were determined using MDA, MAO, and GSH-Px kits, respectively. The results are shown in Table 3. The results showed that the MDA content and MAO activities in the brain and liver of the mice in the AMG were significantly ($p < 0.05$) or very significantly ($p < 0.01$) higher than those of the mice in the CG; however, the GSH-Px activities of mice in the AMG were significantly lower than those of mice in the CG ($p < 0.05$). The MDA content and MAO and GSH-Px activities in the brain and liver of mice in the PSOG did not significantly differ from those of the mice in the CG ($p > 0.05$) but significantly ($p < 0.05$) or very significantly ($p < 0.01$) differed from those of the mice in the AMG.

Effect of peony seed oil on the activities of T-SOD, Cu/Zn-SOD, and Mn-SOD

The activities of T-SOD and Cu/Zn-SOD in the brain and liver of the mice in each group were determined using a SOD typed kit. The activity of Mn-SOD equals the activity of T-SOD minus the activity of Cu/Zn-SOD. The results are shown in Table 4. The results show that the activities of T-SOD, Cu/Zn-SOD, and Mn-SOD in the brain and liver of mice in the AMG were significantly lower than those of mice in the CG ($p < 0.05$). The activities of T-SOD, Cu/Zn-SOD, and Mn-SOD in the brain and liver of mice in the PSOG did not significantly differ from those of mice in the CG ($p > 0.05$) but were significantly higher than those of mice in the AMG ($p < 0.05$).

The results show that the mice in the AMG exhibited obvious senility symptoms. The MDA content and the activity of MAO in the brain and liver of the mice in the AMG significantly increased, and the activities of GSH-Px, T-SOD, Cu/Zn-SOD, and Mn-SOD significantly decreased.

The following findings demonstrated the remarkable anti-aging effects of peony seed oil:

Table 1 Comparison of appearance, behavior, dejecta, and temperament of mice in each group

Item	AMG	CG	PSOG
Appearance	Sparse dull yellow fur	Thick and shiny fur	Thick and shiny fur
Behavior	Reduced activity	Agility and nimbleness	Agility and nimbleness
Ejecta	Thin	Normal	Normal
Temperament	Irritable	Mild	Mild

CG control group, AMG aging model group, PSOG peony seed oil group

Table 2 Summary of the body weight data and food consumption values for mice in each group (n = 10)

Week	1	2	3	4	5	6	
(A) Body weight data (g/mouse)							Increased
CG	34.49 ± 2.12	35.08 ± 3.71	35.23 ± 2.99	35.66 ± 3.32	36.15 ± 3.69	37.27 ± 3.68	2.44 ± 0.43
AMG	32.51 ± 2.36	30.70 ± 2.80	31.05 ± 3.40	32.67 ± 3.67	32.88 ± 3.08	34.25 ± 3.04	1.07 ± 0.22*
PSOG	34.63 ± 3.45	35.63 ± 3.58	35.91 ± 3.49	37.04 ± 3.17	36.65 ± 3.79	38.25 ± 3.02	3.52 ± 0.37* ^{##}
(B) Food consumption values (g/day/mouse)							Total (g/mouse)
CG	5.00 ± 0.39	4.90 ± 0.80	4.94 ± 0.17	4.96 ± 0.83	4.78 ± 0.91	4.70 ± 0.64	204.98 ± 18.00
AMG	3.73 ± 0.67	3.78 ± 0.45	4.50 ± 0.20	4.65 ± 0.24	4.62 ± 0.57	4.73 ± 0.32	182.16 ± 12.9*
PSOG	4.13 ± 0.42	4.68 ± 0.62	4.96 ± 0.62	4.85 ± 0.64	4.51 ± 0.64	4.44 ± 0.73	192.66 ± 14.59

* $p < 0.05$ compared with the CG; ^{##} $p < 0.01$ compared with AMG**Table 3** Comparison of MDA content and MAO and GSH-Px activities in the brain and liver of mice (n = 10)

Group	MDA (nmol/mg prot)		MAO (U/mg prot)		GSH-Px (U/mg prot)	
	Brain	Liver	Brain	Liver	Brain	Liver
CG	0.87 ± 0.10	1.58 ± 0.48	4.93 ± 0.78	2.31 ± 0.41	189.88 ± 19.88	449.50 ± 60.19
AMG	1.18 ± 0.29**	2.18 ± 0.56*	6.14 ± 0.82*	3.75 ± 0.59*	104.78 ± 7.92*	366.30 ± 57.85*
PSOG	0.90 ± 0.16 ^{##}	1.59 ± 0.22 [#]	5.05 ± 0.50 [#]	2.43 ± 0.70 [#]	156.81 ± 29.21 [#]	430.16 ± 40.47 ^{##}

** $p < 0.01$ and * $p < 0.05$ compared with the CG; ^{##} $p < 0.01$; [#] $p < 0.05$ compared with AMG**Table 4** Comparison of the activities of T-SOD, Cu/Zn-SOD, and Mn-SOD in the brain and liver of mice (n = 10, U/mg prot)

Group	T-SOD		Cu/Zn-SOD		Mn-SOD	
	Brain	Liver	Brain	Liver	Brain	Liver
CG	219.37 ± 29.19	125.70 ± 13.61	164.87 ± 16.97	105.53 ± 8.75	54.50 ± 13.61	20.17 ± 10.61
AMG	157.25 ± 14.92*	98.97 ± 3.93*	120.77 ± 15.87*	87.38 ± 6.56*	36.48 ± 10.46*	11.59 ± 6.56*
PSOG	204.18 ± 33.29 [#]	115.80 ± 9.03 [#]	159.54 ± 30.03 [#]	101.67 ± 4.82 [#]	44.64 ± 9.78 [#]	14.13 ± 9.78 [#]

* $p < 0.05$ compared with the CG; [#] $p < 0.05$ compared with AMG

1. Peony seed oil contains many active ingredients, such as paeonol [17], paeoniflorin, and nicotinic acid [32] (see “Introduction” section). According to the fact that peony seeds can use their chemical components to develop and form vigorous seedlings, we considered that peony seed oil may also contain other active

ingredients that can promote material transformation and metabolism and enhance the vitality of organisms. These active ingredients can remove blood stasis; stimulate blood circulation; promote body metabolism; accelerate the breakdown of sugars (D-galactose) and lipids; prevent formation and accumulation of

galactitol, MDA, peroxides, and free radicals; reduce peroxidation; enhance the health of the body; and slow down the aging process.

2. These active ingredients can enhance their anti-aging activities by repressing the expression of MAO and promoting expression of anti-oxidases GSH-Px, CuZn-SOD, and Mn-SOD [17, 33–35]. Studies have shown that a decrease in MAO content may reduce the breakdown of monoamine neurotransmitters, improve brain health, and prevent dementia [28, 29], while an increase in the anti-oxidase can accelerate the removal of free radicals and peroxides and delay aging [36–39].
3. Peony seed oil contains large amounts of α -linolenic acid (up to 40%), which is an essential fatty acid. Research has shown that α -linolenic acid can derive many health care functions, such as inhibiting platelet aggregation, dilating blood vessels, improving blood circulation, and promoting the body's metabolism, by transforming into prostacyclin, eicosapentaenoic acid, and docosahexaenoic acid in the body [40, 41].
4. Peony seed oil contains many antioxidant components that can eliminate free radicals and peroxides through anti-oxidation, prevent lipid peroxidation and the subsequent formation of MDA, and reduce the MDA content in the brain and liver [19, 42].

Peony seed oil can significantly reduce the content or activity of components (such as MDA and MAO) that can cause aging, can significantly increase the activity of anti-oxidases (such as GSH-Px, Cu/Zn-SOD, and Mn-SOD) that have anti-aging functions, and can eliminate the aging effect of D-galactose in mice. Based on these findings, peony seed oil seems to have remarkable anti-aging effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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