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Synergistic antiaging effects of jujube polysaccharide and flavonoid in D-Galactose-Induced aging mice

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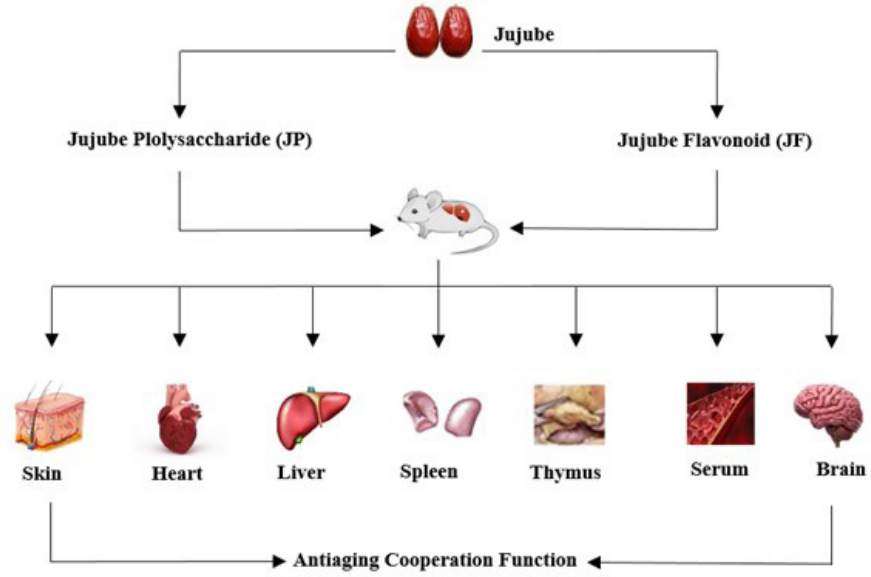
Abstract

In this study, the synergistic effects of jujube polysaccharide (JP) and flavonoid (JF) on aging mice were investigated. Sub-acutely aging mouse models were established using D-galactose. Eighty experimental mice were randomly assigned to normal, model, vitamin C (200 mg/kg), JP (200 mg/kg), JF (200 mg/kg) and JP + JF (low (100 mg/kg), medium (200 mg/kg), and high-dose (300 mg/kg)) groups (10 mice in per group). After 35 days, the physiological aging indices of mice were measured. JP and JF displayed significantly positive synergistic antiaging effects. Compared with an aging model control group, groups administered JP and JF showed increased indices for the thymus and spleen and significantly increased activities of SOD and GSH-PX in the heart, liver, spleen, and brain ($R^2 > 0.99$, $P < 0.01$; $R^2 > 0.98$, $P < 0.01$). The contents of MDA in heart, liver, spleen, brain and serum were significantly decreased ($R^2 > 0.95$, $P < 0.01$), while those of HYP in skin and serum were significantly increased. The concentrations of neurotransmitters (NE, DA, 5-HT, 5-HIAA) in the brain and serum of mice were significantly increased ($P < 0.01$). These results indicated that JP and JF acted not only on relevant tissues or organs of mice, but on all body systems.

Keywords: jujube polysaccharide; jujube flavonoid; antiaging; chinese jujube; red jujube; neurotransmitter enhancement; free radical damage.

Practical Application: This study suggests that JP and JF have considerable synergistic antiaging effects. The results indicate that JP and JF acted not only on relevant tissues or organs of mice, but on all body systems. This work provides a novel theoretical basis for delaying human aging, by preventing and reversing free radical-related diseases.

Graphical Abstract



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1 Introduction

Jujube, also called red or Chinese jujube, belongs to the family *Rhamnaceae*. Jujube is extremely abundant and widely distributed in China (Hou et al., 2019). In terms of area under cultivation, China accounts for over 98% of jujube plants globally, and the areas under jujube cultivation continuously expanding. Jujube is rich in nutrients and has various functional properties. It can be used as food and medicine. The Chinese have a long history of the "drug and food homology" theory (Rashwan et al., 2020). The most representative of the raw materials in Chinese food therapy is jujube. It is found in many traditional Chinese medicine formulas. Furthermore, jujube polysaccharide (JP) and jujube flavonoid (JF) have been reported to exhibit biological properties and pharmacological effects (Ji et al., 2017).

The free radical theory of aging holds that excessive free radicals produced by metabolic processes in the body attack biological macromolecules such as DNA, proteins, and amino acids. Lipid-induced oxidative damage is a major cause of aging and many age-related diseases. Based on this theory, more attention has been paid to the role of dietary antioxidants, which can scavenge free radicals and protect biological macromolecules from oxidative damage. Numerous studies have been conducted on the properties of various natural antioxidants (Chavez- Santiago et al., 2021; Sutedja et al., 2021). For example, many studies have found that JP and JF both scavenge free radicals in the body and increase immunity, thus slowing aging. However, due to the complexity of organisms and the structure of natural substances, the role of natural antioxidants in the human body is complex; hence, there is limited research on the synergy of natural antioxidants (Koley et al., 2016).

The thymus and spleen are vital immune organs, and their indices can reflect the immune function of the body (Ji et al., 2018). Superoxide dismutase (SOD) can prevent oxidation caused by superoxide free radicals (Mu et al., 2017; Qi et al., 2018). The glutathione peroxidase (GSH-PX)-catalyzed decomposition of lipid peroxides supports consenescence delay (Wu et al., 2022). Malondialdehyde (MDA) is a lipid peroxide that can lead to cell dysfunction and even cell death (Li et al., 2021). MDA is an important indicator in measuring body aging. The concentration of hydroxyproline (HYP) in serum can indirectly reflect the metabolism of an organism (Abdelhafidh et al., 2018). Monoamine neurotransmitters, including dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole-acetic acid (5-HIAA), are the important neurotransmitters in the brain (Shergis et al., 2017; Zhao et al., 2015). When the body ages, neurotransmitter concentrations decrease.

In this study, D-galactose (D-gal) was used to induce aging in mice, and the synergistic effects of JP and JF were studied by using thymus and spleen indices, superoxide dismutase and glutathione peroxidase (GSH-PX) activity, and concentrations of malondialdehyde (MDA), hydroxyproline (HYP), and the neurotransmitters NE, DA, 5-HT, and 5-HIAA. This work provides a novel theoretical basis for delaying human aging, by preventing and reversing free radical-related diseases.

2 Materials and methods

2.1 Materials

JP was provided by the Key Laboratory of main grain processing of the Ministry of agriculture, and JF (Purity 70%) was obtained from Nanjing Zelang Medical Technology Co., Ltd. Commercial kits to detect enzyme activation for SOD, GSH-PX, HYP, MDA, DA, NE, 5-HT and 5-HIAA were all purchased from Fusheng Industrial Co., Ltd (Shanghai, China).

2.2 Methods

JP extraction

JP extraction was performed according to the literature (Liu et al., 2020; Rostami & Gharibzahedi, 2016). Jujube was cleaned and denucleated, dried, crushed, and dissolved. Finally, the homogenate was emulsified two times via ultrasonication, and then centrifuged. The supernatant was vacuum concentrated, then underwent 80% alcohol denaturation for 10 h, centrifuged, vacuum dried, exposed to crude polysaccharide and dissolved. The crude extraction was deproteinized by sevag method, preliminarily purified by macroporous resin AB-8 and fractionated by DEAE-52 cellulose column. The components are preliminarily analyzed by Fourier infrared spectrometer, it is proved that the product has the typical characteristics of polysaccharide, including uronic acid, acidic polysaccharide and glucosamine ring structure.

Then the monosaccharide composition was studied by gas chromatography, partial acid hydrolysis, permanganate oxidation and Smith degradation. It was found that the monosaccharide composition was mainly arabinose, xylose, glucose, galactose, mannose and rhamnose. Arabinose mostly existed in the main chain, mannose completely existed in the main chain, and the rest existed in the branch end. The ratio of 1→3, 1→2 and 1→6 glycosidic bonds in polysaccharide is about 14.28:6.02:1. Finality, the conformation of the polysaccharide was studied by iodine potassium iodide reaction analysis and Congo red reaction,It was found that the extracted jujube polysaccharides were non starch polysaccharides. There may be no continuous 1→4 glycosidic bond in their structure, long side chains and more branches, and they had an orderly three strand helical structure.

Animals and experimental design

Eighty male Kunming mice with the body weight of 20 ± 2 g were purchased from the Experimental Animal Centre of Zhengzhou University. The animals were kept at 23 ± 2 °C with a relative humidity of 55 ± 5% and acclimatized in a 14:10 h light/dark cycle. The mice were allowed free access to food and water. Mice were given 3 days to adapt to their environment before the treatments, then weighed.

A total of 80 mice were randomly divided into eight groups: A, normal control group injected subcutaneously with normal saline (NS); and B–H, aging model groups injected subcutaneously with 0.9% D-gal of 0.5 mL of PPP daily. The D-gal aging model groups included the following: B: model control group administered NS for 35 days; C: positive control group administered vitamin C (VC) of 200 mg/kg for 35 days; and D–H:

JP, JF, low-dose JP + JF, medium-dose JP + JF, and high-dose JP + JF groups administered JP (200 mg/kg), JF (200 mg/kg), JP (50 mg/kg) + JF (50 mg/kg), JP (100 mg/kg) + JF (100 mg/kg), and JP (150 mg/kg) + JF (150 mg/kg) for 35 days, respectively.

Organ aging index determination

The mice were weighed 24 h after the final injection. A total of 0.5 g skin tissue per mouse was obtained by daubing 10% sodium sulfide on the backs of the mice. The skin tissues were washed with precooled NS (4 °C), dried with filter paper, and weighed. A total of 0.2 mL of blood was obtained retro-orbitally in 0.4% sodium heparin. The liver, heart, spleen, thymus, and brain were excised from the mice, and subsequently weighed separately. The thymus and spleen indices were calculated according to the following Formula 1 (Li et al., 2017):

$$\text{Thymus or spleen index} = \frac{\text{Thymus or spleen weight}}{\text{Body weight}} \times 100 \quad (1)$$

For biochemical assays, the organ homogenates, including liver, heart, spleen, and brain, were prepared and centrifuged (4000 rpm/min for 10 min) (Tian et al., 2017). The supernatant was collected for further analysis. The samples were subjected to SOD, GSH-PX, MDA, HYP and neurotransmitter level measurements by spectrophotometric methods as described above.

Statistical analysis

The data were presented as mean ± SD and evaluated by one-way ANOVA, followed by the Student's *t*-test to detect the intergroup differences. The differences were statistically significant if $P < 0.05$ and highly significant if $P < 0.01$.

3 Results

3.1 Effects of JP and JF on thymus and spleen indices in aging mice

Figure 1 shows the effects of JP and JF on thymus and spleen indices in the aging mice groups. Compared with the normal control group, the spleen and thymus indices in the aging mice groups significantly decreased ($P < 0.01$, $P < 0.05$). This result indicated that the established aging mice model was established successfully. Compared with the D-gal model control group, the aging mice groups treated with JP, JF and different JP + JF dosages exhibited a significantly positive correlation, and the JP + JF group also demonstrated a dosage-dependent increase in spleen and thymus indices ($P < 0.01$, $P < 0.05$). These results show that JP and JF can resist the aging-related decline in the immune function of the body.

The hierarchy in effectiveness of the JP, JF and medium-dose JP + JF treatments on thymus and spleen indices, was as follows: JP + JF > JF > JP (Table 1). However, the differences were insignificant ($P > 0.05$). These results suggest that JP and JF had synergistic effects on accelerating thymus and spleen injury recovery.

Its mechanism may be because JP can directly improve the amount of T cells, amount of T cell surface receptors and spleen cell proliferation (Jiao et al., 2016). Meanwhile, JF can stimulate lymphocyte proliferation and enhance cellular immune function (Ribeiro et al., 2018). Given the complexity of the organism, JP and JF may have synergistic effects on mice. Nevertheless, the details of the underlying mechanism require further research.

3.2 Effects on antioxidant enzymes in aging mice under the synergistic action of JP and JF

Intervention on SOD activity in different organs of aging mice by synergistic of JP and JF

Figure 2 shows the effects of JP and JF on SOD activity in the aging mice groups. Compared with those in the normal control group, the SOD activities in the brain, heart, liver, and spleen of the aging mice groups significantly decreased ($P < 0.01$, $P < 0.05$). These results indicated that the aging mice model was established successfully. Compared with the D-gal model control group, treating the aging mice groups with VC, JP, JF, and JP + JF at the tested dosages presented a significant difference, and the JP + JF group also displayed a dosage-dependent increase in SOD activity ($P < 0.01$ and $P < 0.05$).

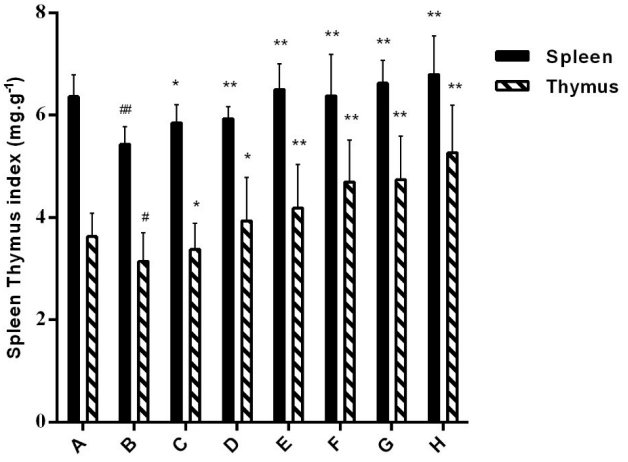

Figure 1. Effects of JP and JF on thymus and spleen indices in aging mice. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and (H) high-dose JP+JF group. #P<0.05, compared with the normal group. ##P<0.01, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

Table 1. Synergistic effects of JP and JF on thymus and spleen indices,

Groups	Dose (mg·[kg·day] $^{-1}$)	Thymus index (mg·g^{-1})	Spleen index (mg·g^{-1})
JP group	200	3.93 ± 0.85^{a}	5.93 ± 0.23^{a}
JF group	200	4.19 ± 0.85^{a}	6.50 ± 0.50^{a}
Medium-dose JP+JF group	100:100	4.74 ± 0.84^{a}	6.63 ± 0.45^{a}

Note: The different superscript letters indicate that within the same columns, values with different superscripts letters differ ($P < 0.05$)

The hierarchy in effectiveness of JP, JF, and medium-dose JP + JF treatments on SOD activity, was as follows: JP + JF > JF > JP, JF > JP ($P < 0.01$), JP + JF > JF ($P > 0.05$) (Table 2). These results indicate that the ability of JF to enhance SOD activity was stronger than that of JP. Hence, JP and JF have synergistic effects on enhancing SOD activity. The mechanism may be ascribed to the fact that JP can not only directly enhance SOD activity but also promote the release of SOD enzymes from the cell surface and block the free radical-induced chain reaction (Lin et al., 2020). For instance, the free radical anti-oxygen activity of fucoidan may be associated with SOD release (Fimbres-Olivarria et al., 2018). JF can also boost SOD activity and activate the production of antioxidant enzymes. As previously mentioned, given the complexity of the organism, JP and JF may have synergistic effects on enhancing the SOD activity in aging mice. For example, JP and JF may exchange electrons with some effective antioxidants, such as vitamin E (VE) in the body, thereby regenerating highly effective antioxidants. This phenomenon can maintain highly effective antioxidants at a threshold level in the body, thereby providing an antiaging function.

Synergistic effect of JP and JF on GSH-PX activity in different organs of aging mice

Figure 3 shows the effects of JP and JF on GSH-PX activity in aging mice. Compared with those of the normal control group, the GSH-PX enzyme activities in the brain, heart, liver, and spleen of the aging mice groups significantly decreased ($P < 0.01$). Compared with the D-gal model control group, treating the aging mice with VC, JP, JF, and JP + JF at the tested dosages exhibited a significant difference, and the JP + JF group also exhibited a dosage-dependent increase in GSH-PX enzyme activity ($P < 0.01$).

The hierarchy of effectiveness of the JP, JF, and medium-dose JP + JF treatments on GSH-PX activity, was as follows: JP + JF > JF > JP, JF > JP ($P > 0.05$) and JP + JF > JF ($P < 0.05$) (Table 3). These results indicated that JP and JF had the synergistic effects on enhancing GSH-PX enzyme activity.

JP and JF can enhance GSH-PX activity, thereby increasing the scavenging capacity for oxygen free radicals. Given the complexity of the organism, JP and JF may have synergistic effects on enhancing GSH-PX enzyme activity in aging mice. Within the oxidation system inside the organism, VC, VE and

Table 2. Synergistic effects of JP and JF on SOD activity in different tissues.

Groups	Dose ($mg\cdot[kg\cdot day]^{-1}$)	Brain ($U\cdot mg^{-1}$)	Heart ($U\cdot mg^{-1}$)	Liver ($U\cdot mg^{-1}$)	Spleen ($U\cdot mg^{-1}$)
JP group	200	7.11 ± 0.71^{a}	7.11 ± 1.44^{a}	7.38 ± 0.73^{a}	7.00 ± 1.16^{a}
JF group	200	10.19 ± 0.49^{b}	10.29 ± 1.19^{b}	10.29 ± 0.78^{b}	10.06 ± 0.92^{b}
Medium-dose JP+JF group	100:100	10.20 ± 0.69^{b}	10.90 ± 0.64^{b}	10.39 ± 0.90^{b}	10.08 ± 1.23^{b}

Note: The different superscript letters indicate that within the same columns, values with different superscripts letters differ ($P < 0.05$).

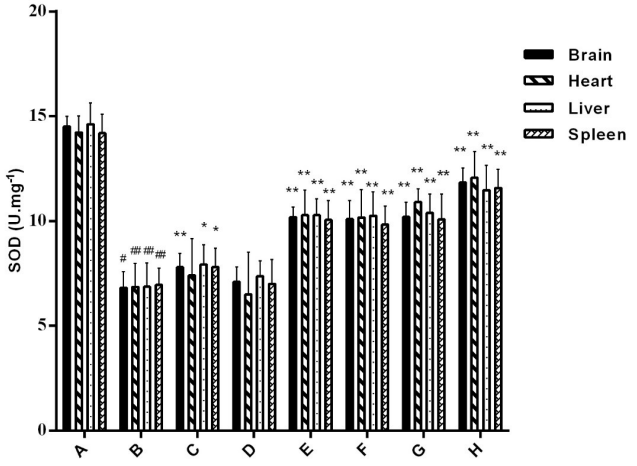

Figure 2. Effects of JP and JF on SOD activity in different organs in aging mice. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and (H) high-dose JP+JF group. #P<0.05, compared with the normal group. ##P<0.01, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

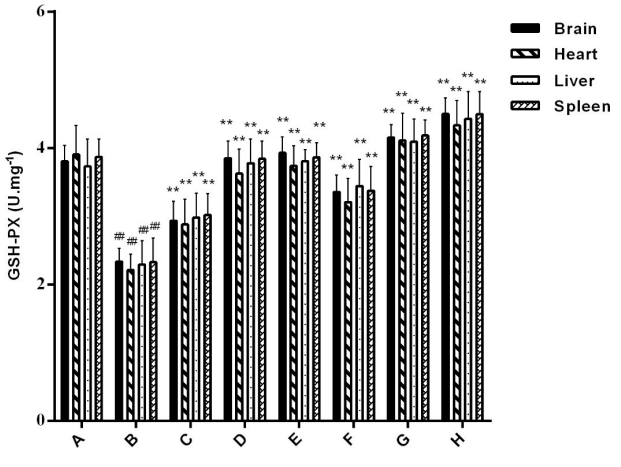

Figure 3. Effects of JP and JF on GSH-PX activity in different organs in aging mice. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and (H) high-dose JP+JF group. #P<0.05, compared with the normal group. ##P<0.01, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

GSH were located in mesenchymal cells, cell membranes, and cells, respectively. VC, VE and GSH act as the first, second, and third lines of antioxidant defense, respectively, thereby forming a protective system to preserve the normal function of the cell (Zhu et al., 2018). Hence, JP and JF may regenerate highly effective antioxidants.

Effect of on HYP concentration in different organs of aging mice by synergy of JP and JF

Figure 4 shows the effects of JP and JF on HYP concentration in different organs in the aging mice groups. Compared with that of the normal control group, the skin and serum HYP concentration in the aging mice groups significantly decreased ($P < 0.01$). Compared with the D-gal model control group, treating the aging mice groups with VC, JP, JF, and JP + JF at the tested dosages exhibited a significant difference, and the JP + JF group also displayed a dosage-dependent increase in HYP concentration ($P < 0.01$).

The hierarchy of effectiveness of the JP, JF, and medium-dose JP + JF groups on HYP concentration, was as follows: JP + JF > JF > JP ($P < 0.05$) (Table 4).

Free radicals can alter collagen molecules, thereby leading to the reduction or loss of the activity of collagenase. Free radicals can also crosslink the hydroxylase macromolecule, preventing proline from becoming HYP, thereby resulting in skin aging (Chen et al., 2016). JP and JF can enhance SOD and GSH-PX enzyme activities. SOD and GSH-PX enzymes can prevent excess free radicals from attacking normal cells and aid in avoiding cellular aging and function loss. Meanwhile, JP and JF may make a highly effective antioxidant, like VE, regenerate (Kou et al., 2015). VE is the membrane protectant of all kinds of biological membranes and can improve HYP concentration.

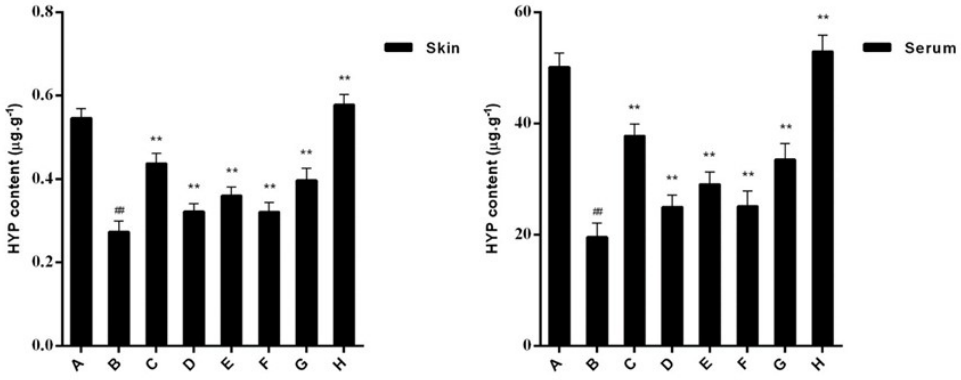

Figure 4. Effects of JP and JF on HYP content in different organs in aging mice. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and (H) high-dose JP+JF group. #P<0.05, compared with the normal group. ##P<0.01, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

Table 3. Synergistic effects of JP and JF on GSH-PX enzyme activity in different tissues.

Groups	Dose (mg·[kg·day]$^{-1}$)	Brain (U·mg^{-1})	Heart (U·mg^{-1})	Liver (U·mg^{-1})	Spleen (U·mg^{-1})
JP	200	3.85 ± 0.25^{a}	3.63 ± 0.36^{a}	3.78 ± 0.35^{a}	3.84 ± 0.26^{a}
JF	200	3.93 ± 0.23^{a}	3.74 ± 0.30^{a}	3.81 ± 0.17^{a}	3.86 ± 0.21^{a}
Medium-dose JP+JF	100:100	4.16 ± 0.19^{b}	4.11 ± 0.40^{b}	4.09 ± 0.33^{b}	4.19 ± 0.22^{b}

Note: The different superscript letters indicate that within the same columns, values with different superscripts letters differ ($P < 0.05$).

Table 4. Synergistic effects of JP and JF on HYP content in different tissues.

Groups	Dose (mg·[kg·day]$^{-1}$)	Skin (μg·g^{-1})	Serum (μg·L^{-1})
JP	200	0.32 ± 0.02^{a}	24.98 ± 2.13^{a}
JF	200	0.36 ± 0.02^{b}	29.06 ± 2.19^{b}
Medium-dose JP+JF	100:100	0.40 ± 0.03^{c}	33.47 ± 2.94^{c}

Note: The different superscript letters indicate that within the same columns, values with different superscripts letters differ ($P < 0.05$).

3.3 Changes of MDA concentrations in different organs of aging mice induced by JP and JF

Figure 5 shows the effects of JP and JF on the MDA concentration in different organs in the aging mice groups. Compared with those of the normal control group, the MDA concentration in the aging mice groups brain, heart, liver, spleen, and serum significantly increased ($P < 0.05$). Compared with the D-gal model control group, treating the aging mice groups with VC, JP, JF, and JP + JF at tested dosages produced a significant difference, and the JP + JF group also displayed the dosage-dependent decrease in the MDA concentration ($P < 0.01$).

The hierarchy of effectiveness of the JP, JF, and the medium-dose JP + JF treatments on MDA concentration, was as follows: JP + JF > JF > JP ($P < 0.05$) (Table 5).

The reason for this phenomenon is that lipid peroxides are generated from unsaturated fatty acids under free radical action. On the one hand, JP and JF may work synergistically to enhance SOD and GSH-PX enzyme activities due to the complexity of the organism. On the other hand, unsaturated fatty acids mainly exist in the biofilm, and JP and JF can regenerate a highly effective antioxidant, such as VE. VE is the membrane protectant of many biological membranes and reduces lipid peroxide generation.

3.4 Changes of neurotransmitter concentration in aging mice treated with JP and JF synergistically

Effects of JP and JF on neurotransmitter concentrations in aging mice brains

Figure 6 shows the effects of JP and JF on neurotransmitter concentration in the brains of the aging mice groups. Compared with that of the normal control group, the neurotransmitter concentration in the brains of the aging mice groups significantly decreased ($P < 0.01$). Compared with the D-gal model control group, treating the aging mice groups with VC, JP, JF, and JP + JF at the tested dosages produced a significant difference, and the JP + JF group also resulted in a dosage-dependent increase in the neurotransmitter concentration in the brain ($P < 0.01$).

Table 5. Synergistic effects of JP and JF on MDA content in different tissues.

Groups	Dose (mg·[kg·day] $^{-1}$)	Brain (mmol·mg^{-1})	Heart (mmol·mg^{-1})	Liver (mmol·mg^{-1})	Spleen (mmol·mg^{-1})	Serum (mmol·mg^{-1})
JP	200	6.51 ± 0.56^{a}	6.11 ± 0.89^{a}	6.63 ± 0.67^{a}	6.48 ± 0.99^{a}	6.58 ± 0.60^{a}
JF	200	4.97 ± 0.59^{b}	4.82 ± 0.64^{b}	5.05 ± 0.61^{b}	5.00 ± 0.55^{b}	5.02 ± 0.47^{b}
Medium-dose JP+JF	100:100	4.81 ± 0.50^{c}	4.39 ± 0.94^{c}	4.22 ± 0.70^{c}	4.39 ± 0.80^{c}	4.50 ± 0.57^{c}

Note: The different superscript letters indicate that within the same columns, values with different superscripts letters differ ($P < 0.05$).

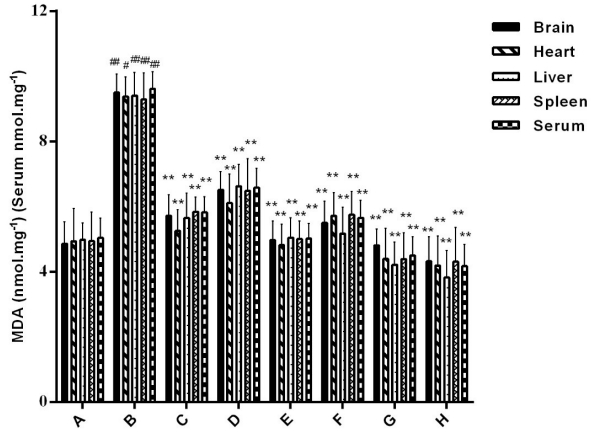

Figure 5. Effects of JP and JF on MDA content in different organs in aging mice. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and H, high-dose JP+JF group. $^{\#}P<0.05$, compared with the normal group. $^{\#\#}P<0.01$, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

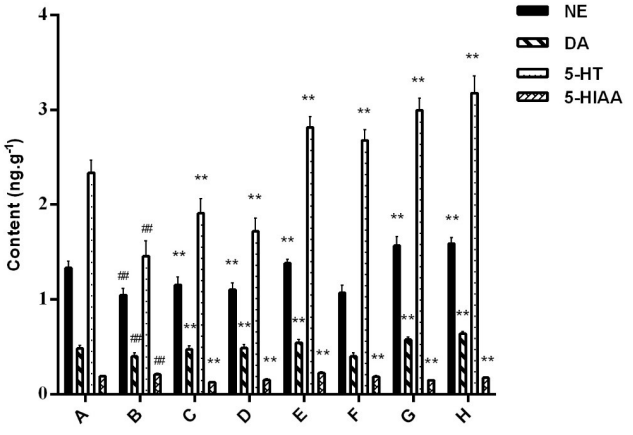

Figure 6. Effects of JP and JF on brain neurotransmitter content in aging mice. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and (H) high-dose JP+JF group. $^{\#}P<0.05$, compared with the normal group. $^{\#\#}P<0.01$, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

Table 6. Synergistic effects of JP and JF on neurotransmitter content in brain.

Groups	Dose (mg·[kg·day]$^{-1}$)	NE (ng·g^{-1})	DA (ng·g^{-1})	5-HT (ng·g^{-1})	5-HIAA (ng·g^{-1})
JP	200	1.10 ± 0 .07^{a}	0.49 ± 0.04^{a}	1.72 ± 0.14^{a}	0.15 ± 0.01^{a}
JF	200	1.38 ± 0.04^{b}	0.54 ± 0.03^{b}	2.82 ± 0.11^{b}	0.19 ± 0.01^{b}
Medium-dose JP+JF	100:100	1.57 ± 0.10^{c}	0.58 ± 0.03^{c}	2.99 ± 0.13^{c}	0.19 ± 0.01^{b}

Table 7. Synergistic effects of JP and JF on serum neurotransmitter content.

Groups	Dose (mg·[kg·day]$^{-1}$)	NE (ng·L^{-1})	DA (ng·L^{-1})	5-HT (ng·L^{-1})	5-HIAA (ng·L^{-1})
JP	200	98.28 ± 8.59^{a}	42.32 ± 3.64^{a}	143.16 ± 12.49^{a}	14.08 ± 0.95^{a}
JF	200	129.10 ± 5.03^{b}	48.44 ± 3.34^{b}	267.49 ± 12.85^{b}	18.13 ± 0.79^{b}
Medium-dose JP+JF	100:100	148.55 ± 9.51^{c}	51.77 ± 3.00^{c}	282.90 ± 14.63^{c}	18.32 ± 1.00^{b}

Note: The different superscript letters indicate that within the same columns, values with different superscripts letters differ ($P < 0.05$).

The hierarchy of effectiveness of JP, JF and medium-dose JP + JF treatments on the neurotransmitter concentration in the brain, was as follows: JP +JF > JF > JP ($P < 0.05$) (Table 6).

The underlying mechanism of the neurotransmitter decrease may be that with aging of the body, the single amine oxidase (MAOB) levels significantly increased which can excessively degrade the levels of neurotransmitters, like NE and DA. Then, the endocrine and nerve functions decline. Previous studies showed that JP can increase the MAOB content, but JF can inhibit the MAOB activity (Damiano et al., 2017; Mubaraki et al., 2017)[,] . However, given the complexity of the organism, JP and JF may have synergistic effects when the neurotransmitter concentration in the brain increases.

Effects of JP and JF on neurotransmitter concentrations in aging mice serum

Figure 7 shows the effects of JP and JF on neurotransmitter concentration in the serum of the aging mice groups. Compared with that in the normal control group, the neurotransmitter concentration in the serum of the aging mice groups significantly decreased ($P < 0.01$). Compared with the D-gal model control group, treating the aging mice groups with VC, JP, JF and JP + JF at the tested dosages exhibited a significant difference, and the JP + JF group also produced a dosage-dependent increase in serum neurotransmitter concentration ($P < 0.01$).

The hierarchy of effectiveness of the JP, JF, and medium-dose JP + JF groups on the neurotransmitter concentration in the serum, was as follows: JP + JF > JF > JP ($P < 0.05$) (Table 7).

This result may be because JP can increase the MAOB concentration, and JF can inhibit the MAOB activity. Thus, JP and JF may have perfect antioxidation and anti-aging effects in vivo, respectively.

3.5 Correlation of all indices in different tissues

Table 8 shows the significant positive correlation between the thymus and spleen indices ($R^2 = 0.85$, $P < 0.01$). These results indicated that the degree of aging in the mice was closely positively related to the condition of thymus and spleen, which together reflect the immune function of the organism.

Table 8. Correlation analyses of thymus and spleen indices.

Analysis sample	Thymus index
Spleen index	0.85**

Note: Statistical significance and highly significant were set at *p < 0.05 and **p < 0.01.

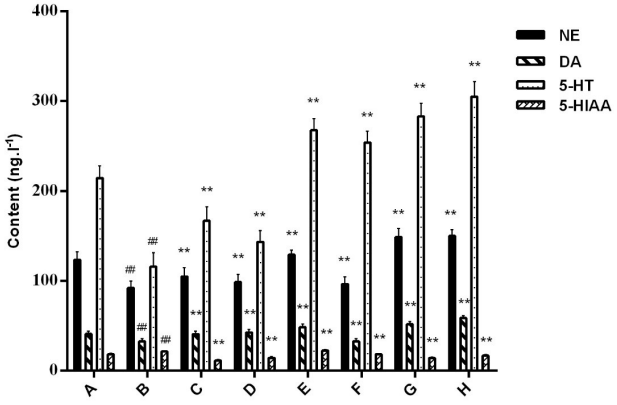

Figure 7. Effects of JP and JF on the neurotransmitter content in aging mice serum. Note: (A) normal control group; (B) D-gal model control group; (C) positive control group; (D) JP group; (E) JF group; (F) low-dose JP+JF group; (G) medium-dose JP+JF group; and (H) high-dose JP+JF group. #P<0.05, compared with the normal group. ##P<0.01, compared with the normal group. *P<0.05, compared with the aging group. **P<0.01, compared with the aging group.

Table 9 shows that the SOD activity in different tissues presented considerable correlation. This result indicated that SOD and GSH-PX enzymes were similar in different aging mice tissues. That SOD enzyme activity was consistent with GSH-PX

Table 9. Correlation of SOF and GSH-PX enzyme activities in different tissues.

Analysis samples		Brain	Heart	Liver
SOD enzyme	Spleen	0.99**	0.99**	0.99**
	Brain		0.99**	0.99**
	Heart			0.99**
GSH-PX enzyme	Spleen	0.99**	0.99**	0.99**
	Brain		0.99**	0.99**
	Heart			0.98**

Note: Statistical significance and highly significant were set at *p < 0.05 and **p < 0.01.

Table 10. Correlation of HYP content in skin and serum.

Analysis samples	Skin
Serum	0.99**

Note: Statistical significance and highly significant were set at *p < 0.05 and **p < 0.01

Table 11. Correlation of MDA content in different tissues.

Analysis samples	Brain	Heart	Liver	Spleen
Serum	0.97**	0.98**	0.98**	0.98**
Brain		0.95**	0.99**	0.99**
Heart			0.95**	0.95**
Liver				0.99**

Note: Statistical significance and highly significant were set at *p < 0.05 and **p < 0.01.

Table 12. Correlation on neurotransmitter content in brain and serum.

Analysis samples		NE	DA	5-HT
Brain	5-HIAA	0.77*	0.61	0.75*
	NE		0.92**	0.86**
	DA			0.74*
Serum	5-HIAA	0.76*	0.61	0.78*
	NE		0.92**	0.82*
	DA			0.70

Note: Statistical significance and highly significant were set at *p < 0.05 and **p < 0.01.

enzyme activity in different mice tissues, supports consistent effects of antioxidant treatment on SOD concentrations in the mice.

Table 10 shows that the HYP concentration in the skin and serum exhibited considerable correlation ($R^2 = 0.99$, $P < 0.01$). This result indicated that HYP concentrations were similar in mice skin and serum. That the skin and serum HYP concentrations were consistent, supports consistent effects of antioxidant treatment on HYP levels in these sites in mice.

Table 11 shows that the MDA content in different tissues presented a considerable correlation ($R^2 > 0.95$, $P < 0.01$).This result indicated that as the mice eating antioxidants, the MDA content in different tissues was consistent.

Table 12 shows that the brain and serum neurotransmitter concentrations displayed considerable correlation ($R^2 > 0.74$, $P < 0.05$; $R^2 > 0.76$, $P < 0.05$). That the brain and serum neurotransmitter concentrations were consistent, supports consistent effects of antioxidant treatment on neurotransmitter levels in these specimens in mice.

4 Conclusion

The results of this study suggest that JP and JF have considerable synergistic antiaging effects. The thymus and spleen indices of mice, SOD and GSH-PX enzyme activity, MDA concentration in examined tissues, HYP concentration in skin and serum, and neurotransmitter concentration of the brain and serum all presented significant correlations.

As demonstrated here, JP and JF not only act on some tissues or organs of mice, but on all body systems. This study lays the foundation for further investigation into the underlying mechanisms of JP and JF antiaging effects and synergy.

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