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马齿苋黄酮抗衰老作用研究

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摘　要:本文研究马齿苋黄酮的抗衰老作用。给药(FPO)6 周后测定血清、肝和脑组织谷胱甘肽过氧化物酶(GSH-Px)和超氧化物歧化酶(SOD)的活力,丙二醛(MDA)的含量及小鼠胸腺和脾脏指数。水迷宫试验与跳台试验测定马齿苋黄酮对衰老小鼠学习与记忆能力的影响。结果显示马齿苋黄酮能显著增加小鼠胸腺和脾脏指数,显著增强血清、肝和脑组织 GSH-Px 和 SOD 活力及显著减少其中 MDA 含量。马齿苋黄酮能显著减少水迷宫试验中小鼠的逃避潜伏期及跳台试验中反应时间与错误次数;显著增加水迷宫试验中小鼠的穿越次数与跳台试验中的记忆潜伏期。马齿苋黄酮有显著的抗衰老作用。

关键词:马齿苋;水迷宫;超氧化物歧化酶;丙二醛;谷胱甘肽过氧化物酶

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Anti-aging Effects of Flavonoids from *Portulaca oleracea* L.

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Abstract: This study was performed to investigate the anti-aging effects of flavonoids from *Portulaca oleracea* L. (FPO). After giving the drug (FPO) for 6 weeks, the effects of FPO on the activities of GSH-Px (glutathione peroxidase), SOD (superoxide dismutase) and the content of MDA (malondialdehyde) in serum, brain and liver tissue were assayed using commercial monitoring kits, and the thymus index and spleen index were also measured. The effects of FPO on learning and memory ability was assessed by Morris water maze and step down test. The results showed that the activities of GSH-Px and SOD in serum, brain and liver tissue were increased significantly by FPO, while the content of MDA was decreased significantly. The thymus index and spleen index were also markedly increased. FPO markedly decreased the escape latency and increased the escape times in water maze test. In step down test, reaction time of learning, as well as error frequency in learning and memory, was markedly decreased by FPO, whereas the latency of memory was markedly increased. These results indicated that FPO had a significant anti-aging effect.

Key words: *Portulaca oleracea* L.; water maze; superoxide dismutase; malondialdehyde; glutathione peroxidase

Introduction

Portulaca oleracea L., a common plant distributing in many parts of the world, is a common "weed" in field crops as well as in turfgrass. It is a kind of traditional ethnodrug herbs as well as a vegetable used for human consumption. It can be eaten cooked as greens or raw as a salad. *P. oleracea* is one of the richest plant sources of α-linolenic acid and omega-3 fatty acid[1]. It provides a rich plant source of nutritional benefits and bioactive ingredients, so that it has been described as a "power food of the future". In areas where this plant is eaten, there is a low incidence of heart disease and cancer [2].

Several native communities use it to treat anemia, arthritis, diarrhoea, fever and vitamin C deficiency. Traditional Chinese Medicine considers that *P. oleracea* has many medical effectiveness, such as heat-clearing and detoxifying, cooling the blood and checking diarrhea. In Saudi Arabia, *P. oleracea* has been used as an antidiuretic, antiseptic and vermifuge in oral ulcer. Recent researches show that *P. oleracea* exhibits a variety of

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biological activities, including antioxidation[3], antidiabetic[4], anticancer[5], anti-inflammatory[6], neuroprotective[7], anti-hypoxic[8], hepatic protection[9] and bacteriostatic action[10].

Flavonoids, a large group of polyphenolic compounds, are abundantly present in herbs and plant-derived foods, such as seeds, vegetables and fruits. It appears to possess a wide rage of biological activities, including antioxidant, anti-inflammatory[11] and anti-aging action[12].

Slow response, poor memory, learning deficits and organ atrophy are the main clinical performances of senescence in human beings. The impaired memory of anile animals has been demonstrated in various learning and memory tests such as Morris water maze and step down test. GSH-Px (glutathione peroxidase), SOD (superoxide dismutase) and MDA (malondialdehyde) are main markers in senescence. Thus, these biomarkers are often used to evaluate the changes in aging process.

Little information has been published regarding the anti-aging activity of *P. oleracea*. The current study aimed to evaluate the anti-aging effects in subacute aging model mice induced by d-galactose, a reducing sugar which is used to induce mimetic aging in experiments[13]. These effects were determined by measuring changes of GSH-Px, SOD and MDA in serum, brain, and liver tissue, organ index (spleen and thymus), as well as the learning and memory ability in Morris water maze and step down test.

Materials and Methods

Drugs and Reagents

The flesh plant, free of obvious defects and blemishes, was collected from the Xiangjiang's bank during August 2014, authenticated as *Portulaca oleracea* L. by Dr Run Tian in Hunan Traditional Chinese Medical College. *P. oleracea* was dried in the shade and then powdered and passed through a 40-mesh sieve. The powder was defatted with diethyl ether and then extracted using 85% aqueous ethanol (ethanol/water: 85/15) twice, 60 min each time. The extraction solution was condensed into liquid extract containing herbal drug 0.5 g/mL. Then the extract was loaded into a glass column with HPD-600 macroporous resin. The column was first eluted with pure water until the eluate becomes colorless, followed by 70% aqueous ethanol. 70% Aqueous ethanol eluent was evaporated to dryness by spray drying, and the drugs was prepared. The total flavonoids content of the drug expressed using rutin was 80.2%, determined using the UV spectrophotometry assay. The flavonoids extract from *P. oleracea* (FPO) was stored for further study. At the time of the experiment, the extract was dissolved with pure water.

D-galactose was purchased from Beijing Chemical-reagent (Beijing, China). Commercial analysis kits of SOD, GSH-Px and MDA were purchased from Jiancheng Institute of Biotechnology (Nanjing, China).

Experimental Animals

Ninety Kunming mice weighing 20 ± 2.0 g were obtained from Laboratory Animal Center of Hunan, China. Animals were kept in a room under standard condition of illumination with a 12 h light-dark cycle at 25 ± 2 ℃ and 55% ± 5% humidity with free access to standard laboratory chow and water. Mice were randomly divided into five groups (Blank group, Aging model group, Aging model + H-FPO group, Aging model + M-FPO group and Aging model + L-FPO group), each group containing 18 animals. The blank group was injected saline (0.5 mL saline/mouse) by subcutaneously back of the neck and received pure water (1 mL pure water/mouse) by oral administration. The aging model group received pure water (1 mL pure water/mouse) by oral administration. The treated groups, Aging model + H-FPO, Aging model + M-FPO and Aging model + L-FPO received FPO orally at a doses of 400, 200 and 100 mg/kg body weight, respectively, for 6 weeks. All animals except for blank group were injected with 5% d-galactose (0.5 mL) subcutaneously into back of the neck for 42 consecutive days.

Statistical Analysis

The obtained datum were presented as means ± standard error. All analyses were performed with SPSS 16.0 software. Significant differences between means were determined by Student's test, and differences at $P < 0.05$ were considered significant.

Step Down Test

After the last administration, the animals were trained in a electric stimulation reflex platform consisted of a 227 × 295 × 105-mm box with a platform (45 mm high × 45 mm diameter) in the left anterior corner of the box. When accommodating the environment of step down apparatus for 5 min, animals were received a 36 v supply voltage. Reaction time during which mice jumped upon the diving platform to avoid electric shock and error frequency of learning (shocking times by electric) in 5 min were recorded as learning ability. 24 h later, the latency, the time during which mice jumped down the platform and error frequency of learning (shocking times by electric) in 3 min were recorded as memory ability.

Morris Water Maze Test

Morris water maze test was performed in a maze pool (50 cm high × 120 cm diameter) with a circular escape platform (28 cm high × 10 cm diameter) in the center of north-east quadrant. The platform was submerged 1.5 cm below the surface of the water (a depth of 30 cm). Water was maintained at a temperature of 21-22 ℃. The pool was divided into 4 equal-sized quadrants (north-east, south-east, north-west and south-west). Before Morris water maze test, all animals had a spatial acquisition phase consisted of 3 days with 2 trials per day, which started at two distinct positions with animals being released facing the sidewall. If a mouse did not reach the platform within 2 min, it would be guided into the goal where it had to remain for 20 s. The next day after the last spatial acquisition day, Morris water maze test was performed. The time that animals reached the platform was recorded as latent period. 10 min later, the escape platform was removed from the maze. The times that animals passed through the escape platform was recorded. After the trial, mice were towel dried and placed in a holding cage under a heating lamp before they were returned to the home cages.

Biochemical Estimations

Animals were weighed after step down test and Morris water maze test. Blood samples were collected from retro-orbital plexus. Then, the animals were cervically dislocated to death. Blood strand for 30 min and then centrifuged to separate serum and stored at-70 ℃ until analysis. Pieces of brain and liver were weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium. The homogenate was centrifuged at 5000 rpm at 4 ℃ for 10 min. The supernatant (10%) was used for biochemical determinations. The spleen and thymus were also weighed.

MDA, SOD and GSH-Px were assayed according to the method that was described by the kits.

Results

Effects of FPO on SOD, GSH-Px Activity and the Level of MDA, Thymus Index and Spleen Index

Effects of FPO on tissue aging induced by d-galactose were evaluated by the activities of antioxidant enzymes such as SOD, GSH-Px and content of Lipid Peroxide, MDA in serum, liver and brain from model and treated rats. Compared to Aging model group, the levels of SOD, GSH-Px, thymus index and spleen index in Aging model + H-FPO group and Aging model + M-FPO group were markedly increased ($P < 0.01$ or $P < 0.05$), while the level of MDA was markedly increased ($P < 0.01$ or $P < 0.05$). These were significant difference in the levels of SOD, GSH-Px, MDA, thymus index and spleen index between Aging model + H-FPO group and Aging model + M-FPO group ($P < 0.01$ or $P < 0.05$). With the increasing of administration, SOD, GSH-Px activity, thymus index and spleen index increased, while the level of MDA decreased (table 1, 2, 3, and 4). Compared to Aging model group, SOD levels in serum, liver, brain of Aging model + H-FPO group were increased by 25.34%, 29.34% and 19.00%, respectively; GSH-Px levels were increased by 15.83%, 43.84% and 29.69%, respectively; While the MDA levels decreased by 18.68%, 39.19%, and 18.57%, respectively; Thymus index and spleen index of Aging model + H-FPO group were increased by 20.79% and 15.94%, respectively.

Effect of FPO on Spatial Memory Performance in water maze test

Spatial memory performance was assessed over successive 3 days using the hidden platform. A probe trial was conducted with no platform on the fourth day (water maze test). The Blank, Aging model + M-FPO group

Table 1 Effect of flavonoids on SOD activity in serum, liver and brain ($n = 18, \bar{x} \pm s$)

Group	Serum (U/mL)	Liver (U/mg)	Brain (U/mg)
Blank	270.52 ± 10.63	355.36 ± 65.27	209.04 ± 11.92
Aging model	192.38 ± 12.15**	240.39 ± 41.78**	138.61 ± 13.43**
Aging model + L-FPO	196.53 ± 11.66**	258.21 ± 40.84**	142.34 ± 12.58**
Aging model + M-FPO	201.26 ± 10.19**#	275.64 ± 48.05**#	148.57 ± 12.16**#
Aging model + H-FPO	241.14 ± 13.88*##ΔΔ	310.92 ± 58.49*##	164.95 ± 12.58**##ΔΔ

Note: compared to blank group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared to Aging model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to Aging model + M-FPO group, $^{\Delta\Delta}P < 0.01$.

Table 2 Effect of flavonoids on GSH-Px activity in serum, liver and brain ($n = 18, \bar{x} \pm s$)

Group	Serum (U/mL)	Liver (U/mg)	Brain (U/mg)
Blank	265.74 ± 28.63	116.61 ± 14.32	151.73 ± 17.46
Aging model	211.57 ± 23.85**	70.53 ± 9.06**	89.83 ± 11.95**
Aging model + L-FPO	214.84 ± 22.39**	73.46 ± 7.88**	94.66 ± 12.09**
Aging model + M-FPO	228.05 ± 24.63**#	84.82 ± 9.14**##	104.28 ± 13.94**##
Aging model + H-FPO	245.06 ± 25.47*##Δ	101.45 ± 11.37**##ΔΔ	116.50 ± 16.43**##Δ

Note: compared to Blank group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared to Aging model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to Aging model + M-FPO group, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$.

Table 3 Effect of flavonoids on MDA content in serum, liver and brain ($n = 18, \bar{x} \pm s$)

Group	Serum (nmol/mL)	Liver (nmol/mg)	Brain (nmol/mg)
Blank	4.46 ± 0.15	3.29 ± 1.22	14.78 ± 0.79
Aging model	6.53 ± 0.61**	9.16 ± 2.83**	20.46 ± 0.83**
Aging model + L-FPO	6.20 ± 0.59**	8.94 ± 2.57**	19.95 ± 0.78**
Aging model + M-FPO	6.13 ± 0.44**#	7.03 ± 2.24**#	17.23 ± 0.79**##
Aging model + H-FPO	5.31 ± 0.36**##ΔΔ	5.57 ± 1.86**##Δ	16.66 ± 0.67**##Δ

Note: compared to Blank group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared to Aging model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to Aging model + M-FPO group, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$.

Table 4 Effect of FPO on thymus and spleen index ($n = 18, \bar{x} \pm s$)

Index (mg/g)	Blank	Aging model	Aging model + L-FPO	Aging Model + M-FPO	Aging model + H-FPO
Thymus	2.28 ± 0.21	1.78 ± 0.23**	1.82 ± 0.24**	1.96 ± 0.19**#	2.15 ± 0.24##Δ
Spleen	4.52 ± 0.29	3.89 ± 0.26**	3.95 ± 0.31**	4.33 ± 0.27*##	4.51 ± 0.32##

Note: compared to Blank group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared to Aging model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to Aging model + M-FPO group, $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$.

and Aging model + H-FPO group rapidly learned the location of the platform. Compared to Aging model group, the latency in Blank group, Aging model + M-FPO group and Aging model + H-FPO group was markedly decreased whereas traversing times was markedly increased ($P < 0.01$ or $P < 0.05$). The latency in Aging model + H-FPO was decreased by 37.06%, whereas traversing times was increased by 101.19%. There were significant difference between Aging model + M-FPO group and Aging model + H-FPO group ($P < 0.05$). FPO tended to decrease the escape latency and increase the escape times in water maze test. These results suggested that FPO administration prevent d-galactose induced deficits in spatial memory (table 5).

Effect of FPO on learning and memory ability in step down test

Learning and memory ability were assessed by step down test. The Blank group, Aging model + M-FPO group and Aging model + H-FPO group rapidly learned

Table 5 Spatial performance memory of water maze test ($n = 18, \bar{x} \pm s$)

Index (mg/g)	Blank	Aging model	Aging model + L-FPO	Aging Model + M-FPO	Aging model + H-FPO
Latency (sec)	26.14 ± 10.76	55.82 ± 14.13 **	49.38 ± 13.76 **	45.55 ± 12.28 $^{**\#}$	35.13 ± 11.41 $^{*\#\#\Delta}$
Traversing times (s)	8.29 ± 1.95	3.73 ± 1.38 **	3.95 ± 1.42 **	5.37 ± 1.66 $^{**\#\#}$	6.78 ± 1.73 $^{*\#\#\Delta}$

Note: compared to Blank group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared to Aging model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to Aging model + M-FPO group, $^{\Delta}P < 0.05$.

and memorized to avoid the electric shock. Compared to Aging model group, the reaction time and error frequency of learning and memory in Blank group, Aging model + M-FPO group and Aging model + H-FPO group were markedly decreased whereas the latency (stepping down the platform) was markedly increased ($P < 0.01$ or $P < 0.05$). The reaction time and error frequency of learning or memory in Aging model + H-FPO group were decreased by 26.59%, 40.92%, and 50.41%, respectively, whereas latency of memory was increased by 42.39%. Except latency, there were significant difference in the learning and memory index between Aging model + M-FPO group and Aging model + H-FPO group ($P < 0.05$). FPO tended to strengthen the awareness of avoiding injury. These results suggested that FPO administration prevent d-galactose induced deficits in learning and memory ability (table 6).

Table 6 Learning and memory of step down test ($n = 18, \bar{x} \pm s$)

Group	Learning		Memory	
	Reaction time (sec)	Error frequency (s)	Latency (sec)	Error frequency (s)
Blank	20.14 ± 4.12	3.12 ± 1.43	135.54 ± 37.16	1.51 ± 0.82
Aging model	54.98 ± 9.76 **	7.38 ± 2.15 **	77.35 ± 25.02 **	3.63 ± 1.59 **
Aging model + L-FPO	50.83 ± 8.43 **	6.94 ± 1.92 **	79.28 ± 25.87 **	3.16 ± 1.28 **
Aging model + M-FPO	44.57 ± 7.19 $^{**\#\#}$	5.59 ± 1.78 $^{**\#}$	101.63 ± 28.76 $^{**\#}$	2.63 ± 1.12 $^{**\#}$
Aging model + H-FPO	40.36 ± 5.05 $^{**\#\#\Delta}$	4.36 ± 1.52 $^{*\#\#\Delta}$	110.14 ± 30.39 $^{*\#\#}$	1.80 ± 1.03 $^{\#\#\Delta}$

Note: compared to Blank group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared to Aging model group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared to Aging model + M-FPO group, $^{\Delta}P < 0.05$.

Discussion and Conclusion

It is believed that oxidative stress is an important pathogenic factor in human chronic diseases, such as the aging process, atherosclerosis and related vascular diseases, and neurodegeneration[14], so it is a needed therapeutic method to reduce oxidative stress in the overall therapy of senescence. MDA, lipid peroxidative product, promotes peroxidation damage of tissues in the body. The content change of MDA represents the strength of lipid reaction, indirectly indicating the severity of the cell damage caused by free radicals. The mammalian brain has various defensive mechanisms against oxidative stress, such as GSH-Px and SOD, scavengers of free radicals, which plays an important role in the process of antioxidant defenses.

D-galactose is commonly used to prepare senescence model. It has been reported that repeated injecting d-galactose could induce senescent symptoms in animals, such as reduction in immunological activity, abnormal alterations in biochemistry markers, abnormity expressions in gene, lags in response, planotopokinesia and memory impairments.

In this present study, chronic injection of d-galactose induced a significant decrease of SOD and GSH-Px activity, and an increase of MDA level in serum, liver, and brain, indicating that there was oxidative damage in the mice. We measured the activity of SOD and GSH-PX, and MDA levels in liver, serum and brain, as well as learning and memory ability to understand the mechanism of FPO on d-galactose induced senescence. The results showed that d-galactose-treated mice had shortened latencies and increased numbers of errors in the step down test. FPO (400 or 200 mg/kg) significantly improved memory impairment and learning ability. The improvement by FPO on learning and memory deficits

was accompanied by promoting SOD and GSH-Px activity and declining MDA level. From these findings, it was suggested that the FPO against the oxidative damage in the brain and liver. The anti-oxidative system may be mediated by increasing SOD and GSH-Px activity, and MDA contents. These results are in agreement with the findings reported by other investigators who showed that memory is impaired by oxidative stress[15]. Morris water maze and step down test are one of the most widely used tasks in studying the neural mechanisms of spatial learning and memory and avoidance of danger. During the test, the groups treated with FPO (400 and 200 mg/kg) markedly improved its deficits in working memory induced by D-galactose. aging is characterized by an initial loss of memory and slow response. Therefore, the improved memory observed in FPO-treated mice deserves special emphasis in evaluating therapeutical effect of senescence. The datum obtained from the Morris water maze and step down test suggested that FPO could be having a high efficacy against learning, memory and action dysfunction.

In conclusion, the findings in this study suggested that FPO protected the mice from the d-galactose induced senescence. It was also indicated that the ameliorating effects of FPO on aging might be related to activating the antioxidant system.

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