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Original Article

The antioxidant effect of *Asparagus cochinchinensis* (Lour.) Merr. shoot in D-galactose induced mice aging model and *in vitro*

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Abstract

Background: An increasing number of plant components and their extracts have been shown to have beneficial health effects in humans. We aimed to explore the antioxidant effects of the aqueous extract of *Asparagus cochinchinensis* (Lour.) Merr. shoot *in vivo* and *in vitro*.
Methods: A total of 80 Kun Ming mice were randomly divided into four groups (20/group). The mice in the control group received a daily subcutaneous injection of saline. A daily injection of D-galactose was administered to the aging model group, the vitamin C (Vc) group (positive control group), and the extract treatment group. Regular measurement of blood cells, nitric oxide synthase (NOS), catalase (CAT) activities, superoxide dismutase (SOD) activities, nitric oxide (NO), and malondialdehyde (MDA) concentration, and the expressions of *NOS*, *SOD*, and glutathione peroxidase (*GPX*) in serum levels were obtained. Furthermore, the microstructure of mice viscera was observed using hematoxylin and eosin staining.
Results: The aqueous extract of *A. cochinchinensis* (Lour.) Merr. had similar 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH·) [or 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+)] and higher hydroxyl radicals (or superoxide anion; $p < 0.05$) radical scavenging capabilities to Vc. Moreover, compared with the aging model group, the aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot could obviously increase NOS, CAT, and SOD activities and the NO content, and reduce the MDA content ($p < 0.05$). Additionally, the microstructure of mice viscera was obviously improved and the expressions of *NOS, SOD and GPX* were also manifestly increased in the treatment group ($p < 0.05$).
Conclusion: The aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot had a strong radical scavenging capability *in vivo* and *in vitro*, and might be used to diminish radicals in the body and consequently prevent aging.

Keywords: antioxidant; *Asparagus cochinchinensis* (Lour.) Merr.; enzyme activity; gene expression; shoot

1. Introduction

Aging is the combined result of physiological and pathological activities. The body increasingly shows the effects of enhanced oxidation and reduced antioxidant activity during the process of aging.[1] An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate oxidation chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.[2] Although the accumulation of oxygen radicals plays a negative biological role in aging,[3] natural antioxidants have been proven to be

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successfully used as *in vivo* scavengers of oxygen radicals to protect the cardiovascular and cerebrovascular systems, resist cancer, and delay aging.[4,5]

Various traditional Chinese medicines, such as wolfberry flower,[6] *Rubus alceaefolius* Poir,[7] and Okra leaf[8] are demonstrated potential resources for natural antioxidants. *Asparagus cochinchinensis* (Lour.) Merr. is a genus in the plant family Liliaceae. Previous studies indicate that *A. cochinchinensis* (Lour.) Merr. not only has antibacterial and anticancer effects,[9,10] but also has an antioxidant effect *in vivo*.[11–13] However, the impacts of *A. cochinchinensis* (Lour.) Merr. on the expression of antioxidant enzymes, as well as on histological and pathological changes, remain unclear. The D-galactose model has been successfully used for screening antiaging drugs and health products, because a large amount of D-galactose administration can result in a series of pathological and physiological changes related to oxidative stress.[14] In the present study, the effects of *A. cochinchinensis* (Lour.) Merr. shoot on radical scavenging were investigated. The effects of aqueous extract on the nitric oxide synthase (NOS), catalase (CAT), and superoxide dismutase (SOD) activities, as well as the nitric oxide (NO) and malondialdehyde (MDA) content in organs, were examined based on the D-galactose-induced aging mouse model. This study aimed to systematically elucidate the antioxidant mechanism of shoot of *A. cochinchinensis* (Lour.) Merr. aqueous extract, and provide scientific evidence for further applications of *A. cochinchinensis* (Lour.) Merr.

2. Methods

2.1. Pharmaceutical preparation

The pharmaceutical preparation was performed according to those steps described in Zhang et al.[11] A total of 20 g of powdered shoots of *A. cochinchinensis* (Lour.) Merr. were extracted in 160 mL water, and thereafter boiled and extracted three times (1 h/time). The three extracts were combined, filtered, and concentrated using a rotating evaporator to obtain the aqueous extract of *A. cochinchinensis* (Lour.) Merr. The aqueous extract of the shoot of *A. cochinchinensis* (Lour.) Merr. was extracted strictly in accordance with Chinese pharmacopoeia. Each preparation was undertaken by one specific person and strictly controlled. In addition, liquid chromatography was conducted to certify that the aqueous extract from each preparation had similar major compositions. Meanwhile, the dried extract yield obtained from the original materials was approximately 8%. Then, the aqueous extract was dissolved in distilled water for stocking extract solution (0.7 g/mL, frozen for use).

2.2. Measurement of radical scavenging in vitro

1,1-Diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH·) has been effectively used to monitor chemical reactions involving radicals, which was commonly used in antioxidant assays.[15] The measurement of DPPH·scavenging was performed as described previously.[16] Briefly, 2 mL of extract solution was quickly mixed with 2 mL 1.25×10^{-4} mol/L DPPH, and placed in the dark at room temperature for 30 minutes. Subsequently, the absorbance at 517 nm was measured. Ethanol was used as the negative control, while vitamin C (Vc) was used as the positive control. The DPPH·scavenging rate was determined using $D\% = [1 - (Ai - Aj)/Ac] \times 100\%$ (Ai, the absorbance after the extracts were added; Aj, the basal absorbance; Ac, the negative control absorbance). The measurement of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}\cdot$) scavenging was performed as described previously.[17] The $ABTS^{+}$ scavenging rate was determined as follows: $D\% = (1 - Ai/0.7) \times 100\%$ [Ai, the absorbance at 734 nm after the *A. cochinchinensis* (Lour.) Merr. extracts were added]. Furthermore, the measurements of hydroxyl radicals (OH) and superoxide anion were performed using a kit developed by the Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) based on the Fenton reaction. The Fenton reaction is the most common chemical reaction for the generation of hydroxyl radicals, which is proportional to the amount of superoxide anion. Typically, the Griess reagent turns red when an electron acceptor is given. There was a proportional relationship between the color depth and the amount of OH. The absorbance value was detected at 510 nm by using the scientific microplate reader (Thermo Multiskan Spectrum, Vantaa, Finland).

2.3. Animals used for the antioxidant capability of A. cochinchinensis (Lour.) Merr

A total of 80 male Kun Ming mice (2 months old, weighing 20 ± 2 g) for laboratory use were obtained from the Xiangya Medical School of Central South University (Changsha, China). The mice were housed three per cage under constant environmental conditions (20–24°C; 12 hour light-dark cycle) and were given *ad libitum* access to standard pelleted food and water. This study was carried out in strict accordance with the recommendations in the national guidelines for the use of animals in scientific research "Regulations for the Administration of Affairs Concerning Experimental Animals". The protocol was also approved by Xiangya Hospital, School of Medical Central South University (permit number 2008-0002). All surgeries were rapidly performed under diethyl ether anesthesia, and all efforts were made to minimize suffering.

2.4. Aging and drug treatment models' construction

The mice were randomly divided into four groups (20mice/group) including a control group, an aging model group, a Vc group (positive control), and an extract treatment group. For the aging model group, the Vc group, and the extract treatment group, all mice received daily a subcutaneous injection of D-galactose at a dose of 100 mg/kg for aging model construction. For mice in the control group, an equal volume of physiological saline was injected. After that, mice in the Vc positive control group received daily intragastric administration of Vc, while mice in the extract treatment group received prepared aqueous extract at the dose of 200 mg/kg of body weight. In

addition, the control group and the aging model group mice all received intragastric administration of the same amount of distilled water. The mice were fed separately according to the group (without water limitation, 30 consecutive days).

2.5. Blood and pathological tissue sample preparation

At 24 hours after the final drug administration, 20 μL blood samples from the mice eyeballs were quickly transferred into heparin sodium treated 5 mL centrifuge tubes containing dilution solution (Jingrui Ltd., Shenzhen, China). The red blood cell (RBC), hemoglobin concentration (HGB), platelets (PLT), and white blood cell (WBC) were measured using a KT6180 autocytometer (Genius Electronics Co., Ltd., Shenzhen, China). The rest of the blood was placed in heparin sodium-treated Eppendorf tubes (Bio-Rad, Hercules, CA) (5 μL 2 U/uL heparin was used to wet the tube wall), and then centrifuged at 900 g/min for 10 minutes. The supernatant was then utilized to measure other indicators.

The mice were sacrificed, followed by rapid isolation of the livers, kidneys, hearts, and brains. The tissues were fixed in Bouin's solution for 24 hours, and stored in 70% ethanol. The tissues underwent the following steps in order: gradient ethanol dehydration, dimethylbenzene transparency, paraffin embedding, slicing with Leica RM2255 microtome (Leica, Nussloch, Germany) (5–7 um), regular Hematoxylin-eosin (HE) staining, and neutral resin sealing. Finally, the sections were observed and photographed under a microscope (Motic Group Co., Ltd., Xiamen, China). All of the experiments were conducted with six random samples for each group.

2.6. Measurements of the NOS, SOD, and CAT activities, NO and MDA contents

Tissues (0.3–0.6 g) were processed using cold saline to prepare 1% homogenate (for SOD, NO, CAT, and protein) and 10% homogenate (for MDA and total NOS). After centrifugation at 300 g/min for 5 minutes, the supernatants were used for measurement of these indicators using kits developed by the Jiancheng Bioengineering Institute (Nanjing, China). All of the experiments were conducted with six random samples for each group.

2.7. Semiquantitative reverse transcriptase-polymerase chain reaction

Total mRNA of tissue was isolated using an RNA extraction kit (Ambiogen Life Science Technology Ltd., Changsha, China). The cDNA was synthesized using a first strand cDNA synthesis kit (Tiangen Biotech Co., Ltd., Beijing, China). Polymerase chain reaction amplification was conducted according to Xie et al.[18] Primers used for the present study were: *SOD*, 5′-ACGAAGGGAGGTGGATGCTG-3′ and 5′-ACGGT TGGAGGCGTTCTGCT-3′; *NOS*, 5′-TTGGAGCGAGTTGT GGATTG-3′ and 5′-TGAGGGCTTGGCTGAGTGA-3′; glutathione peroxidase (*GPX*), 5′-GCCTGGATGGGGAGAAGATA-3′ and 5′-GCAAGGGAAGCCGAGAACTA-3′; and *actin*, 5′-GAGACCT TCAACACCCCAGC-3′ and 5′- ATGTCACGCA CGATTTCCC -3′. All of the experiments were conducted with six random samples for each group.

2.8. Statistical analysis

All the experiment data were expressed as mean ± standard deviation, and statistical evaluation was performed using the SPSS 18.0 package program (SPSS Inc., Chicago, IL, USA). One-way analysis of variance followed by *post hoc* multiple comparisons with the Limited Slip Differential (LSD) test was used to compare the differences among the groups for the effects of *A. cochinchinensis* (Lour.) Merr. on blood components, activities of NOS, CAT, SOD, and contents of NO and MDA. The Student *t* test was used to analyze the difference between groups for the radical scavenging activities of *A. cochinchinensis* (Lour.) Merr. A p value < 0.05 was considered to be a statistically significant difference.

3. Results

3.1. Radical scavenging in vitro of shoot of A. cochinchinensis (Lour.) Merr.

Our results showed that the extract of shoot of *A. cochinchinensis* (Lour.) Merr. (2.0 g/mL) had similar DPPH·and $ABTS^{+}$·scavenging capabilities, but obviously higher OH ($p < 0.01$) and superoxide anion ($p < 0.05$) scavenging capabilities than Vc *in vitro* (Table 1). These results indicated that the extract of *A. cochinchinensis* (Lour.) Merr. shoot had a strong radical scavenging capability *in vitro*.

3.2. The antioxidant capability of the shoot of A. cochinchinensis (Lour.) Merr. by hemogram analysis

After treatment with D-galactose, there was a significant decrease ($p < 0.05$) of WBC in the aging group compared with that in the control group, indicating that the defense system was inhibited and the aging model was successfully constructed. Compared to the aging model group, the WBC in the extract treatment group was significantly higher

Table 1
Radical scavenging activities of *Asparagus cochinchinensis* (Lour.) Merr. ($n = 6$ for each group).

	DPPH·(%)	$ABTS^{+}$·(%)	OH (U/mL)	Superoxide anion (U/L)
Vc group	43.52 ± 2.18	35.36 ± 2.14	24.31 ± 1.25	76.39 ± 2.30
Extract treatment group	34.43 ± 2.12	34.53 ± 2.42	46.34 ± 3.420**	80.11 ± 6.65*

Measurements were repeated 10 times; data are expressed as mean ± standard deviation; Student *t* test was used to analyze the difference between groups.
*$p < 0.05$ and **$p < 0.01$ compared with the Vc control group.
$ABTS^{+}$ = 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic); DPPH· = 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; OH = hydroxyl radicals; Vc = vitamin C.

($p < 0.05$), indicating that the *A. cochinchinensis* (Lour.) Merr. extract promoted the immunity of the blood. The RBC number and HGB level in the aging model group were significantly lower than those in the negative control group, but increased to the comparative level in the extract treatment group compared with that in the control and Vc groups ($p < 0.05$). Similarly, an increased blood PLT number in the aging model group was significantly downregulated by the application of *A. cochinchinensis* (Lour.) Merr. extract to a similar level as that in the Vc group ($p < 0.05$, Table 2).

3.3. Effects of aqueous extract of A. cochinchinensis (Lour.) Merr. shoot on enzyme activities of antioxidant system

The *A. cochinchinensis* (Lour.) Merr. extract significantly improved the NOS, CAT, and SOD activities *in vivo*. As shown in Table 3, the activities of SOD, NOS, and CAT were significantly decreased in the aging group as compared with those in the control group ($p < 0.05$). Meanwhile, the activities of SOD, NOS, and CAT were restored to high levels by the aqueous extract of shoot of *A. cochinchinensis* (Lour.) Merr. treatment in kidney, brain, liver, serum, and heart samples ($p < 0.05$). The effects of extract and Vc on the activities of NOS, CAT, and SOD were similar and without statistical difference ($p > 0.05$).

3.4. Effects of aqueous extract of A. cochinchinensis (Lour.) Merr. shoot on content of NO and MDA

Decreasing levels of NO and increasing MDA were found in the aging group compared with the control group ($p < 0.05$). The aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot could significantly promote the NO content and reduce their MDA content. Compared to the aging model group, the NO content of the extract treatment group was significantly higher ($p < 0.05$) in the brain, liver, serum, heart, and kidney. Meanwhile, the NO content of the brain and kidney of the extract treatment group was significantly higher ($p < 0.05$) than that of the control group. Compared to the aging model group, the MDA content of the extract treatment group was significantly decreased in the brain, liver, serum, heart, kidney, and lung ($p < 0.05$). Meanwhile, the MDA content in the brain, serum, and kidney of the extract treatment group was significantly lower ($p < 0.05$) than that of the control group. Furthermore, the data revealed that the NO contents of the extract treatment group were similar to that of the Vc group in each organ, and the MDA contents of the extract treatment group were similar to that of the Vc group in brain, serum, and kidney (Table 4).

3.5. Effect of aqueous extract of A. cochinchinensis (Lour.) Merr. shoot on the microstructure of mice viscera

The results of tissues stained with hematoxylin and eosin are shown in Fig. 1. The tissues of liver, kidney, lung, brain,

Table 2
Effects of *Asparagus cochinchinensis* (Lour.) Merr on blood components ($n = 6$ for each group).

Treatment	WBC/10^9/L	RBC/10^{12}/L	HGB/g/L	PLT/10^9/L
Negative control group	8.13 ± 0.47[a]	11.71 ± 1.24[a]	169.12 ± 11.89[a]	469.21 ± 44.25[c]
Aging model group	4.20 ± 0.49[c]	6.52 ± 0.61[b]	114.10 ± 43.90[b]	615.14 ± 85.33[a]
Vc group	5.11 ± 0.53[b]	11.12 ± 1.30[a]	155.21 ± 6.60[a]	452.18 ± 44.02[b]
Extract treatment group	5.16 ± 0.44[b]	10.23 ± 0.81[a]	148.21 ± 13.68[a]	478.45 ± 48.43[b]

Data are expressed as the mean ± standard deviation; one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons using the Limited Slip Differential (LSD) test was used to analyze the difference among groups.
[a,b,c] Values with different letters showed significant difference for the same indicator among different groups ($p < 0.05$).
HGB = hemoglobin concentration; PLT = platelets; RBC = red blood cell; Vc = vitamin C; WBC = white blood cell.

Table 3
Effects of shoot of *Asparagus cochinchinensis* (Lour.) Merr. on the activities of nitric oxide synthase (NOS), catalase (CAT), and superoxide dismutase (SOD) ($n = 6$ for each group).

	Treatment	SOD/U/mg prot	NOS/U/mg prot	CAT/U/mg prot
Brain	Control group	98.31 ± 4.44[a]	1.48 ± 0.04[a]	32.34 ± 0.98[b]
	Aging model group	63.99 ± 4.20[b]	1.35 ± 0.06[b]	20.67 ± 2.16[c]
	Vc group	100.36 ± 5.01[a]	1.59 ± 0.05[a]	48.11 ± 2.17[a]
	Extract treatment group	90.41 ± 5.93[a]	1.40 ± 0.08[a]	38.54 ± 2.57[a]
Liver	Control group	55.65 ± 2. 81[b]	0.84 ± 0. 13[b]	54.61 ± 3. 32[a]
	Aging model group	49.54 ± 2. 88[c]	0.74 ± 0. 12[c]	45.72 ± 4. 51[b]
	Vc group	59.75 ± 3.89[a]	1.24 ± 0.31[a]	56.18 ± 4.38[a]
	Extract treatment group	54.22 ± 3.97[a]	0.98 ± 0.16[a]	50.67 ± 3.87[a]
Serum	Control group	86.42 ± 9. 29[b]	40.52 ± 3.32[b]	0.31 ± 0.08[a]
	Aging model group	72.58 ± 6.47 [c]	33.76 ± 4.51[c]	0.12 ± 0.013[b]
	Vc group	96.97 ± 7.87 [a]	47.55 ± 3.69[a]	0.34 ± 0.06[a]
	Extract treatment group	84.78 ± 7.56[a]	45.34 ± 5.09[a]	0.26 ± 0.02[a]
Heart	Control group	51.58 ± 2.96[a]	0.51 ± 0.06[a]	5.06 ± 0.75[a]
	Aging model group	33.96 ± 2.19[c]	0.26 ± 0.05[b]	1.53 ± 0.38[b]
	Vc group	42.87 ± 1.89[b]	0.54 ± 0.07[a]	5.01 ± 0.54[a]
	Extract treatment group	39.63 ± 3.16[b]	0.40 ± 0.07[a]	2.54 ± 0.43[a]
Kidney	Control group	56.24 ± 4.57[b]	1.76 ± 0. 27[a]	14.30 ± 1. 34[b]
	Aging model group	50.87 ± 5.09[c]	1.51 ± 0. 14[b]	5.24 ± 1. 06[c]
	Vc group	63.77 ± 5.31[a]	1.68 ± 0.15[a]	17.65 ± 1.87[a]
	Extract treatment group	55.61 ± 3.98[a]	1.56 ± 0.10[a]	12.70 ± 1.52[a]
Lung	Control group	60.87 ± 5.98[b]	1.84 ± 0.21[b]	16.38 ± 1.87[a]
	Aging model group	49.78 ± 5.34[c]	1.54 ± 0.16[c]	7.98 ± 0.85[b]
	Vc group	69.78 ± 7.68[a]	2.11 ± 0.27[a]	18.74 ± 2.31[a]
	Extract treatment group	72.36 ± 8.54[a]	2.34 ± 0.35[a]	17.65 ± 2.57[a]

Data were expressed as the mean ± standard deviation; one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons with the Limited Slip Differential (LSD) test was used to analyze the difference among groups.
[a,b,c] Values with different letters showed significant difference for the same indicator among different groups ($p < 0.05$).
Vc = vitamin C; prot = protein.

Table 4
The effects of *Asparagus cochinchinensis* (Lour.) Merr. on the contents of nitric oxide (NO) and malondialdehyde (MDA) ($n = 6$ for each group).

	Treatment	NO/μmol/L	MDA/U/mg prot
Brain	Control group	12.50 ± 1.06^{b}	1.43 ± 0.09^{b}
	Aging model group	5.08 ± 0.85^{c}	2.59 ± 0.25^{a}
	Vc group	26.36 ± 1.89^{a}	0.89 ± 0.03^{c}
	Extract treatment group	20.35 ± 0.98^{a}	1.23 ± 0.08^{c}
Liver	Control group	4.95 ± 0.17^{b}	1.12 ± 0.27^{b}
	Aging model group	1.91 ± 0.16^{c}	1.55 ± 0.14^{a}
	Vc group	5.57 ± 0.36^{a}	0.98 ± 0.11^{c}
	Extract treatment group	4.23 ± 0.02^{a}	1.39 ± 0.10^{b}
Serum	Control group	907.64 ± 46.14^{b}	24.96 ± 3.80^{b}
	Aging model group	503.26 ± 27.08^{c}	29.64 ± 4.46^{a}
	Vc group	987.89 ± 51.27^{a}	21.87 ± 3.14^{c}
	Extract treatment group	879.62 ± 31.54^{a}	25.67 ± 4.09^{c}
Heart	Control group	4.08 ± 0.92^{a}	0.39 ± 0.078^{b}
	Aging model group	1.10 ± 0.24^{c}	2.53 ± 0.35^{a}
	Vc group	3.77 ± 0.44^{b}	0.36 ± 0.08^{b}
	Extract treatment group	3.23 ± 0.34^{b}	2.36 ± 0.56^{a}
Kidney	Control group	6.12 ± 0.62^{b}	2.24 ± 0.39^{b}
	Aging model group	3.09 ± 0.27^{c}	4.90 ± 0.51^{a}
	Vc group	12.66 ± 1.45^{a}	1.69 ± 0.49^{c}
	Extract treatment group	9.56 ± 1.24^{a}	2.05 ± 0.70^{c}
Lung	Control group	7.25 ± 0.36^{b}	2.78 ± 0.31^{b}
	Aging model group	4.58 ± 0.54^{c}	5.17 ± 0.63^{a}
	Vc group	11.28 ± 1.10^{a}	2.03 ± 0.31^{c}
	Extract treatment group	10.36 ± 0.98^{a}	2.47 ± 0.25^{b}

Note: Data were expressed as mean ± standard deviation; one-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons with the Limited Slip Differential (LSD) test was used to analyze the difference among groups.
[a,b,c] Values with different letters showed significant difference for the same indicator among different groups ($p < 0.05$).
Vc = vitamin C; prot = protein.

and heart were observed. These tissues in the control group showed cardiac muscle fibers cell bodies extending to a shuttle shape, a trend of parallel arranging, assembling into a beam, structure integrated, intercellular boundaries clear, packing closely, gradation distinct, clearly visible band, and intercalated disc. However, these tissues in the aging model group showed cardiac muscle fiber plumping, structure fuzzy and twisted shortening, significantly widened interval, and obvious capillary vessel of myocardial interstitial congestion. After aqueous extract of shoot of *A. cochinchinensis* (Lour.) Merr. treatment, the microstructures of mice viscera were obviously improved, which was similar to those of the Vc group. These results indicated that D-galactose induced changes in the morphology, number of hepatocytes, and neurons. Meanwhile, the *A. cochinchinensis* (Lour.) Merr. extract has protecting effects on the microstructure of mice viscera.

3.6. Semiquantitative analysis of the A. cochinchinensis (Lour.) Merr. shoot extracts

Semiquantitative analysis showed that the expressions of *NOS*, *SOD*, and *GPX* were all obviously lower in the aging group compared with those in the control group. Meanwhile, the expressions of *NOS*, *SOD* and *GPX* in the serum of extract treatment group were similar to that in the Vc group, but obviously higher than those in the aging group. The expression levels of *NOS* and *GPX* in the extract treatment group were higher than those in the control group (Fig. 2A). Furthermore, the expression levels of *NOS*, *SOD*, and *GPX* in the liver of extract treatment group were similar to those in the control group, which were higher than those in the aging group and lower than those in the Vc group (Fig. 2B). The expression levels of *NOS*, *SOD*, and *GPX* in kidney in the extract treatment group were similar to those in the control and Vc groups, which, however, were obviously higher than those in the aging group (Fig. 2C).

4. Discussion

Aging is one of the most common and highest risk factors for most human diseases, including neurodegeneration, cancer, and metabolic syndrome. As an effective intervention, natural antioxidants play an important role in aging. In the present study, the mechanism of the natural antioxidant *A. cochinchinensis* (Lour.) Merr. in the aging process was revealed by premature aging in a mouse model. The results showed that the aqueous extract of *A. cochinchinensis* (Lour.) Merr. not only had a strong radical scavenging ability, but also increased the gene expressions of *SOD* and *GPX*, as well as the activity of SOD and CAT. Moreover, the aqueous extract of *A. cochinchinensis* (Lour.) Merr. increased the NO content, reduced the MDA content, and played an important role in pathological changes.

Studies on the biology of aging have proven that the development of interventions for the possible slowing of aging is inevitable.[19] In fact, the accumulation of oxygen radicals plays a negative biological role in aging.[3] Radicals can cause all kinds of oxidative damage involved in aging.[20] Previous studies have indicated that natural substances can not only inhibit radical production,[21] but also indirectly prohibit radical-caused damage through enhancing the activity of antioxidant enzymes.[22] MDA is a peroxide product of lipids, and changes in the MDA content can directly indicate the peroxidation rate of the myocardial cell membrane, and reflect the production of oxygen radicals.[23] SOD can catalyze the disproportionation reaction of the superoxide radicals and reduce MDA production. The reduction of SOD activity is directly related to the membrane damage caused by radicals and their metabolic products.[24] CAT is a very important defensive antioxidant enzyme which can degrade the peroxide hydrogen production during SOD-mediated disproportionation of superoxide radicals. GPX is the first selenium-containing enzyme and can catalyze the reduction of hydroperoxides through GSH and consequently degrade the reactive oxygen species (ROS) to reduce oxidative stress. In the present study, the aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot showed a strong oxidant scavenging activity, and could significantly increase the SOD and CAT activities, increase the expressions of *SOD* and *GPX*, and reduce the MDA content. These results indicated that the aqueous extract could reduce the radicals through promoting the expression and activity of antioxidant enzymes.

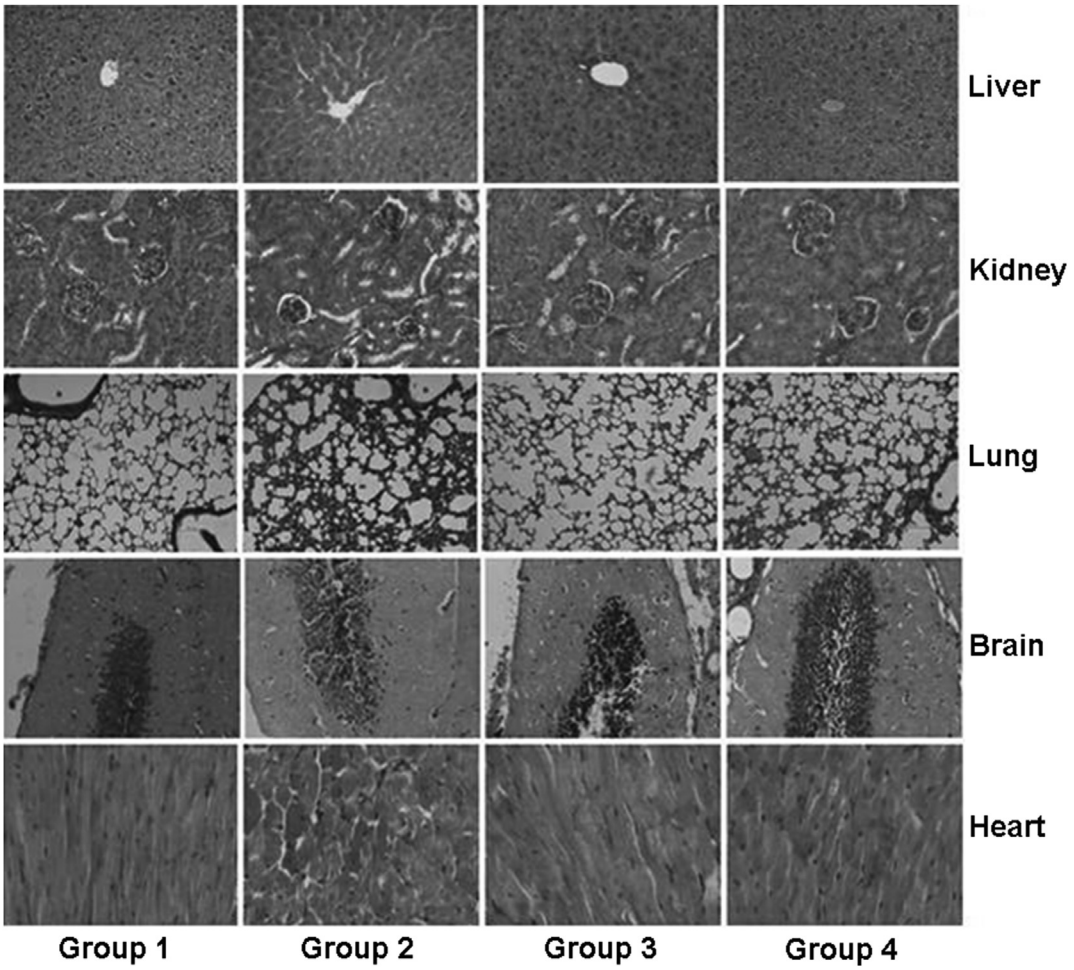

Fig. 1. The different tissues stained with hematoxylin and eosin obtained from D-galactose-induced senile mice: Group 1—the control group; Group 2—the aging model group; Group 3—the vitamin C (Vc) group; Group 4—the extract treatment group.

NO is produced from L-arginine by NOS,[25] which is the rate-limiting enzyme for NO production.[26] The reduction of NOS activity can decrease the NO content, and consequently cause aging.[27] In this study, *NOS* expression and NO content were upregulated in mice treated with *A. cochinchinensis* (Lour.) Merr. aqueous extract. Thus, the aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot might promote the antioxidant activity through enhancing *NOS* expression and NO content. Previous studies indicate that NO can promote the antioxidant activity of the body through participating in a

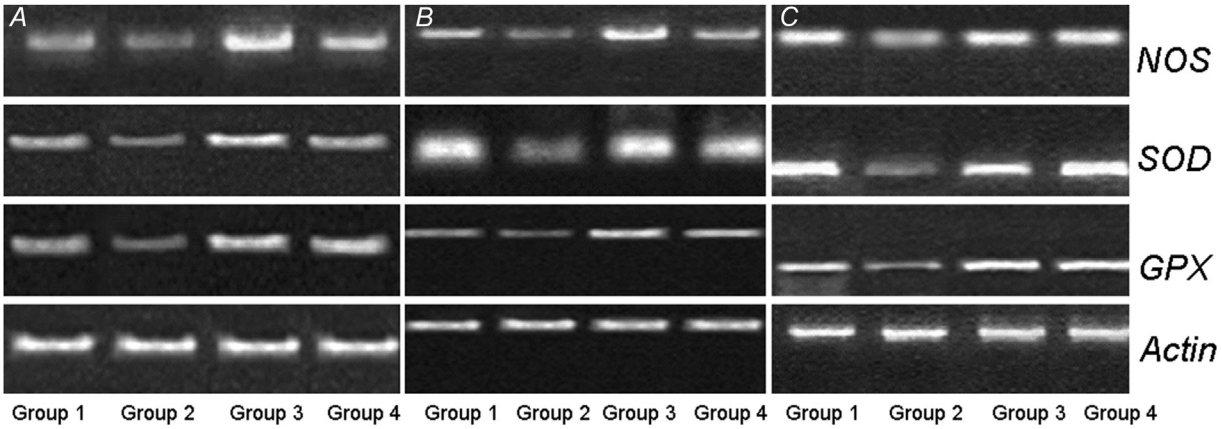

Fig. 2. Expressions of genes of nitric oxide synthase (NOS), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in different tissues: (A) blood; (B) liver; (C) lung; Group 1—the control group; Group 2—the aging model group; Group 3—the vitamin C (Vc) control group; Group 4—the extract treatment group.

series of physiological processes, such as endothelial cell (or smooth muscle cell) regulation, inflammation, tissue damage, respiration, digestion, circulation, and immunity.[28,29] Meanwhile, the high expression level of NO might induce superoxide anion toxicity and result in aging.[30−32] Considering that the microstructures of mice viscera were obviously improved in the extract treatment group, we speculated that *A. cochinchinensis* (Lour.) Merr. shoot extract might delay the aging process by enhancing the antioxidant activity which was related to the NO content.

In conclusion, the antioxidant effect of the aqueous extract of *A. cochinchinensis* (Lour.) Merr. shoot was available through enhancing antioxidant enzyme expression, increasing NOS, CAT, and SOD activities and the NO content, and reducing the MDA content. However, the active ingredients of *A. cochinchinensis* (Lour.) Merr. shoot were still unclear, which needed to be identified and thereafter studied in the future.

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