

Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice

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Abstract

Oxidative damage of biomolecules increases with age and is postulated to be a major causal factor of various physiological function disorders. Consequently, the concept of anti-age by antioxidants has been developed. *Lycium barbarum* fruits have been used as a traditional Chinese herbal medicine and the data obtained in *in vitro* models have clearly established the antioxidant potency of the polysaccharides isolated from the fruits. In the present study, the age-dependent changes in the antioxidant enzyme activity, immune function and lipid peroxidation product were investigated and effect of *Lycium barbarum* polysaccharides on age-induced oxidative stress in different organs of aged mice was checked. *Lycium barbarum* polysaccharides (200, 350 and 500 mg/kg b.w. in physiological saline) were orally administrated to aged mice over a period of 30 days. Aged mice receiving vitamin C served as positive control. Enzymatic and non-enzymatic antioxidants, lipid peroxides in serum and tested organs, and immune function were measured. Result showed that increased endogenous lipid peroxidation, and decreased antioxidant activities, as assessed by superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant capacity (TAOC), and immune function were observed in aged mice and restored to normal levels in the polysaccharides-treated groups. Antioxidant activities of *Lycium barbarum* polysaccharides can be comparable with normal antioxidant, vitamin C. Moreover, addition of vitamin C to the polysaccharides further increased the *in vivo* antioxidant activity of the latter. It is concluded that the *Lycium barbarum* polysaccharides can be used in compensating the decline in TAOC, immune function and the activities of antioxidant enzymes and thereby reduces the risks of lipid peroxidation accelerated by age-induced free radical.

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1. Introduction

A major characteristic of an aging organism is its progressive functional decline, including a loss of adaptive responses to stresses, with the passage of time (Ian and Grotewiel, 2006). One currently major cause of aging is the concept of oxidative stress as a root of aging (Golden and Melov, 2001). Oxidative stress is described generally as a condition under which increased production of free radicals, reactive species (including singlet oxygen and reactive lipid peroxidation products, such as reactive aldehydes and peroxides), and oxidant-related reactions occur that result in damage.

Current studies suggest that development of anti-aging drugs from Chinese medicinal herbs may be one of the possible interventions (Chang, 2001; Bastianetto and Quirion, 2002; Lei et al., 2003). Oriental herbal medicine has been widely investigated for drug development because it has fewer side effects (Wong et al., 1994). *Lycium barbarum* belongs to the plant family Solanaceae. Red-colored fruits of *Lycium barbarum* have been used as a traditional Chinese herbal medicine for thousands of years (Gao et al., 2000). The earliest known Chinese medicinal monograph documented medicinal use of *Lycium barbarum* around 2300 years ago. *Lycium barbarum* fruits have a large variety of biological activities and pharmacological functions and play an important role in preventing and treating various chronic diseases, such as diabetes, hyperlipidemia, cancer, hepatitis, hypo-immunity function, thrombosis, and male infertility (Gao et al., 2000; Li, 2001). It is well recognized that free

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radical scavengers or antioxidants plays a important role in slowing down biological aging (Andrès et al., 2006; Linnane and Eastwood, 2006). The evidence suggests that *Lycium barbarum* is effective to be an anti-aging agent as well as nourishment of eyes, livers and kidneys. The anti-aging property of *Lycium barbarum* is found in the polysaccharides isolated from the red-colored fruits and has been investigated in different models (Qi et al., 2001; Peng et al., 2001; Wang et al., 2002; Gan et al., 2003, 2004; Zhang et al., 2005). For example, extracts of *Lycium barbarum* have anti-decrepit effect in brain and heart tissues in mice by increasing the activity of superoxide dismutase (SOD) (Xu and Fang, 2000). The extracts can still prolongs the life span of *Drosophila* (Xu, 2003). Polysaccharides isolated from *Lycium barbarum* fruits exhibit anti-aging function in fruit flies and mice (Wang et al., 2002). Although numerous studies have been published on humans and animals examining the health aspects of *Lycium barbarum* polysaccharides, to our knowledge, there have been scarce studies to investigate its beneficial effects on health from the aspect of its antioxidant activity in vivo.

Therefore, in the present study, we investigated age-dependent changes in the activity of antioxidant enzymes and the immune function in the mice studied and assess the regulatory effects of polysaccharides isolated from *Lycium barbarum* fruits on oxidative stress in aged mice to improve the understanding of the health benefits of these polysaccharides.

2. Methods and materials

2.1. Preparation of polysaccharides

Fruits of *Lycium barbarum*, family *solanaceae*, originated from china were purchased from JingHe county herb market (Xinjiang, China), and identified by Professor D.S. Chen, School of Traditional Chinese Medicine, Xinjiang University. Voucher specimens (HYT-PM040008) were preserved in XinJiang Natural Product Research Institute.

Polysaccharides from *Lycium barbarum* was prepared by the method of Luo et al. (2004). The dried fruit samples (100 g) were ground to fine powder and put in 1.5 l of boiling water and decocted for 2 h by a traditional method for Chinese medicinal herbs. The decoction was left to cool at room temperature, filtered and then freeze-dried to obtain crude polysaccharides. The dried crude polysaccharides were refluxed three times to remove lipids with 150 ml of chloroform:methanol solvent (2:1) (v/v). After filtering the residues were air-dried. The result product was extracted three times in 300 ml of hot water (90 °C) and then filtered. The combined filtrate was precipitated using 150 ml of 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and vacuum-dried, giving desired polysaccharides (13 g).

The content of the polysaccharides was measured by phenol-sulfuric method (Masuko et al., 2005). Result showed that the content of the polysaccharides in the extract may reach 97.54%.

2.2. Determination of in vivo antioxidant activity of the polysaccharides

2.2.1. Animals grouping and treating

Sixty 20-month-old, body weight 28–40 g, aged Kunming mice and ten 3-month-old, body weight 19–26 g, young Kunming mice were provided by Laboratory Animal breeding Center attached to our institute. The animals were kept under controlled conditions (temperature: 23 ± 2 °C; humidity: $55 \pm 5\%$; 14 h light–10 h dark cycle) using an isolator caging system (Niki Shoji, Co., Tokyo) and allowed free access to standard laboratory pellet diet and water throughout the experimental period. All experimental animals were overseen and approved by the Animal Care and Use Committee of XinJiang Medical University before and during experiments.

Aged Kunming mice were randomly divided into six groups (10 for each): Group II (the aged control), Group III, Group IV, Group V, Group VI and Group VII. Young Kunming mice (Group I) served as normal control. The polysaccharides and vitamin C were administered orally to test animals using vehicle solution (physiological saline) by using a gastric gavage.

- Group I: Normal control mice were maintained on standard laboratory pellet diet and water ad libitum, without administering medicine for 30 consecutive days.
- Group II: Aged control mice were maintained on standard laboratory pellet diet and water ad libitum, without administering medicine for 30 consecutive days.
- Group III: Aged mice received polysaccharides (200 mg/kg b.w.) in appropriate volumes of physiological saline by using gastric gavage and allowed free access to standard laboratory pellet diet and water for 30 consecutive days.
- Group IV: Aged mice received polysaccharides (350 mg/kg b.w.) in appropriate volumes of physiological saline by using gastric gavage and allowed free access to standard laboratory pellet diet and water for 30 consecutive days.
- Group V: Aged mice received polysaccharides (500 mg/kg b.w.) in appropriate volumes of physiological saline by using gastric gavage and allowed free access to standard laboratory pellet diet and water for 30 consecutive days.
- Group VI: Aged mice received medicine (polysaccharides plus vitamin C; 1/1) (500 mg/kg b.w.) in appropriate volumes of physiological saline by using gastric gavage and allowed free access to standard laboratory pellet diet and water for 30 consecutive days.
- Group VII: Aged mice received vitamin C (500 mg/kg b.w.) in appropriate volumes of physiological saline by using gastric gavage and allowed free access to standard laboratory pellet diet and water for 30 consecutive days.

After overnight fasting following the last drug administration, the animals were sacrificed by decapitation. Blood samples

were harvested, kept at -20°C until analyzed. Blood samples were centrifuged at 4000 rpm for 3 min at 4°C and the serum was separated. The serum MDA level was measured. The organs (including liver, heart, brain, kidney and lung) were removed, weighed and homogenized immediately with DY89-II homogenizer (NingBo Scientz Biotechnology Co., Ltd.) fitted with teflon plunger, in ice chilled 10% KCl solution (10 ml/g of tissue). The suspension was centrifuged at $671 \times g$ at 4°C for 10 min and clear supernatant was used for the following estimations of activity of SOD, CAT, GSH-Px, TAOC, and levels of MDA by spectrophotometric methods. Spleen and thymus were removed and kept frozen at -80°C until measurement.

2.2.2. Analytical methods

Superoxide dismutase activity was measured according to the method described by Misra and Fridovich (1972) based on the inhibition of auto-oxidation of epinephrine by SOD at 480 nm in a LKB Ultraspec-2 spectrophotometer. Tissue homogenate (0.5 ml) was diluted to 1.5 ml with distilled water, and 250 μl of chilled ethanol and 100 μl of chilled chloroform were added. The mixture was shaken and centrifuged. The supernatant was used for the assay of enzyme activity. 1.5 ml of the supernatant was added to 1.5 ml of 0.1 mol/l carbonate–bicarbonate buffer, pH 10.2, containing 0.3 mmol/l EDTA. The contents were mixed, and the reaction was initiated by adding 200 μl of epinephrine (pH 3.0, 3 mmol/l) to the buffered reaction mixture. The change in optical density per minute was measured at 480 nm. The enzyme activity was expressed as unit per milligram of protein, where 1 U is defined as the enzyme concentration required to inhibit 50% of epinephrine auto-oxidation in 1 min under the assay conditions.

The assay of Beers and Sizer (1952) was used to measure CAT. Substrate solution for CAT was 59 mM H_2O_2 in 50 mM potassium phosphate buffer at pH 7.0. Assays were initiated by the addition of 0.1 ml of supernatant to 1.9 ml of deionized water and 1 ml of substrate solution. The disappearance of H_2O_2 was measured as the decrease in absorbance at 240 nm. Catalase activity was expressed as U/mg protein (1 U is the amount of enzyme that utilizes 1 μmol of hydrogen peroxide/min).

The GS-Px activity of the supernatant was determined spectrophotometrically at 423 nm. The reaction mixture was composed of GSH, distilled water, and the supernatant. The reaction was stopped by adding trichloroacetic acid. The content of residual GSH was then measured using 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB). One unit of GS-Px activity was defined as 1 μmol /l GSH consumption per minute (Liu and Ng, 2000).

Lipid peroxidation (LPO) was measured by high performance liquid chromatography (Shimadzu LC 10A, Shimadzu, Kyoto, Japan) as described previously (Nielsen et al., 1997). The photodiode array detector (Shimadzu SPD-M10AVP) and a C18 column (4.6 $\mu\text{m} \times 25.5 \mu\text{m}$ Shimpack HRC-ODS) were used for assay. After pretreatment with thiobarbituric acid, the sample was injected and an isocratic elution was carried out with a mobile phase consisting of 60:40 (v/v) 10 mmol/l potassium dihydrogen phosphate (pH 6.80):methanol. MDA was detected

at a wavelength of 532 nm. Peak authenticity was confirmed by use of pure 1,1,3,3-tetraethoxypropane standards. The malondialdehyde (MDA) content of the samples was expressed as nmol MDA formed/mg protein.

Total antioxidant capacity (TAOC) was measured on an Olympus AU-600 analyzer using the TAOC kit (Medikon SA, Gerakas, Greece) as described previously (Kampa et al., 2002). Briefly, antioxidants in the sample inhibit the bleaching of crocin from 2,2-azobis-(2-amidinopropane) dihydrochloride (ABAP) to a degree that is proportional to their concentration. The assay was performed at 37°C in the following steps: 2 μl of sample, calibrator or control were mixed with 250 μl of crocin reagent (R1) and incubated for 160 s. Subsequently, 250 μl of ABAP (R2) were added and the decrease in absorbance at 450 nm was measured 26 s later. Values of TAOC were expressed as U/mg protein.

Lipofuscin (LPF) contents were determined by the method of Vernet et al. (1988) and Hill and Womersley (1991). For lipofuscin measurement, 0.5 ml of tissue homogenate was suspended in 3 ml of isopropanol and 2 ml of chloroform. This was allowed to stand for 30 min and centrifuged at $1800 \times g$ in a refrigerated centrifuge. The fluorescence was measured using a spectrofluorimeter with extinction at 360 nm and emission at 440 nm. Lipofuscin concentrations were expressed as $\mu\text{g/g}$ tissue.

The phagocytic activity of neutrophils in whole blood was conducted as per the method described by Panasiuk et al. (2005). Briefly, standard strain of *Staphylococcus aureus* was procured from the Division of Standardization (IVRI). Eighteen hours culture was opsonised with pooled mice serum in an incubator for 1 h. An equal volume (500 μl) of PMNs and 500 μl opsonised bacterial suspension was incubated at 37°C for half an hour, maintaining PMNs and bacteria at a 1:5 ratio. Thereafter, it was stained with 500 μl of Acridine orange stain (0.015%, Sigma, St. Louis, MO, USA), vortexed and centrifuged at 4°C , 13000 rpm to obtain cell pellet. Finally, 500 μl crystal violet was added and centrifuged as above. The pellet was resuspended in cold sterile PBS (500 μl) and wet mount seen under ultraviolet source with excitation filter of 530 nm. Phagocytic activity, expressed by the percentage of phagocytosed neutrophil in 100 cells and phagocytic index, determined on the unit of Staphylococci ingested by single PMNs, was counted in 100 cells.

The spleen and thymus of the mice were also removed and weighed to obtain the index of the spleen and thymus. The thymus and spleen indices were assayed according to the method of Zhang et al. (2003) and calculated according to the following formula: Thymus or spleen index = weight of thymus or spleen/body weight $\times 100$.

2.3. Statistical analyses

All data in table are expressed as mean \pm S.D. ($n=10$) and differences between groups were assessed by analysis of variance (ANOVA) and Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$. All statistical analyses were carried out using SPSS for Windows, Version 11.5 (SPSS, Chicago, IL).

3. Results

3.1. Effect of the *Lycium barbarum polysaccharides* on antioxidant enzymes activity in lungs in aged mice

As shown in Table 1, there was significant difference in SOD activities, MDA level, TAOC observed in lung between the aged control and young mice control ($P < 0.05$) but not in GSH-Px, and CAT activity. Declined antioxidant enzymes activity (SOD, CAT activity, TAOC) or increased lipid peroxidation product (MDA) in aged tissues (groups III–VII) were significantly elevated or reduced with administration of polysaccharides and vitamin C in a dose-dependent manner. The antioxidant activity of polysaccharides is stronger than that of vitamin C at identical dose of 500 mg/kg b.w.

3.2. Effect of the *Lycium barbarum polysaccharides* on antioxidant enzymes activity in livers in aged mice

As was shown in Table 1, significantly decreased antioxidant enzymes activity (SOD, GSH-Px, CAT, TAOC) and increased

MDA level were observed in livers in aged control mice compared with normal control ($P < 0.01$). Administration of polysaccharides and vitamin C dose-dependently increased the activity of antioxidant enzymes, reduced the level of MDA in livers in aged mice (groups III–VII). Moreover, the inhibition by polysaccharide administration of age-induced oxidation is stronger than that of vitamin C at identical dose of 500 mg/kg b.w.

3.3. Effect of the *Lycium barbarum polysaccharides* on antioxidant enzymes activity in hearts in aged mice

A marked increase in MDA production and decrease of antioxidant enzymes activity (SOD, GSH-Px, CAT, TAOC), were observed in hearts of aged control mice (Table 1) when compared with normal control ($P < 0.01$). Polysaccharides and vitamin C treatment significantly inhibited the formation of MDA in mice hearts and raised antioxidant enzymes activity in a dose-dependent manner (groups III–VII). Likewise, polysaccharides exhibited stronger antioxidation effects than vitamin C at identical dose of 500 mg/kg b.w.

Table 1

Effect of polysaccharides on activities of SOD (NU/mg protein), CAT (U/mg protein), GSH-Px (U/mg protein), TAOC (U/mg protein) and levels of MDA (nmol/mg protein or/ml serum) in tested organs in aged mice

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Lung							
SOD	11.41 ± 1.13	10.18 ± 0.77 ^c	9.98 ± 0.58 ^b	12.98 ± 0.60 ^b	13.99 ± 1.21 ^b	15.52 ± 0.78 ^b	11.59 ± 1.64 ^a
CAT	4.82 ± 0.57	4.52 ± 0.47	4.46 ± 0.46	4.68 ± 0.64	5.05 ± 0.56 ^a	5.47 ± 0.61 ^b	4.74 ± 0.39
GSH-Px	3.11 ± 0.61	2.77 ± 0.39	3.98 ± 0.47 ^b	4.43 ± 0.33 ^b	5.92 ± 0.53 ^b	6.21 ± 0.65 ^b	3.91 ± 0.43 ^b
TAOC	1.13 ± 0.09	0.96 ± 0.06 ^c	1.04 ± 0.14	1.32 ± 0.14 ^b	1.49 ± 0.13 ^b	1.71 ± 0.09 ^b	1.30 ± 0.13 ^b
MDA	2.34 ± 0.49	2.85 ± 0.34 ^c	2.37 ± 0.23 ^b	2.20 ± 0.27 ^b	2.03 ± 0.24 ^b	1.87 ± 0.21 ^b	2.51 ± 0.17 ^a
Liver							
SOD	8.70 ± 0.67	7.58 ± 0.66 ^d	8.45 ± 0.38 ^b	9.45 ± 1.15 ^b	15.88 ± 1.06 ^b	18.17 ± 0.94 ^b	14.02 ± 0.97 ^b
CAT	2.14 ± 0.20	1.65 ± 0.19 ^d	1.80 ± 0.11 ^a	1.92 ± 0.15 ^b	2.15 ± 0.17 ^b	2.86 ± 0.40 ^b	1.86 ± 0.16 ^a
GSH-Px	10.91 ± 0.92	8.78 ± 0.81 ^d	10.41 ± 0.81 ^b	12.69 ± 1.18 ^b	14.24 ± 1.07 ^b	17.62 ± 1.53 ^b	10.97 ± 0.84 ^b
TAOC	2.01 ± 0.19	0.84 ± 0.11 ^d	1.16 ± 0.19 ^b	1.36 ± 0.13 ^b	1.87 ± 0.28 ^b	2.44 ± 0.41 ^b	1.48 ± 0.16 ^b
MDA	13.46 ± 0.96	15.64 ± 0.64 ^d	14.53 ± 0.66 ^b	12.57 ± 1.11 ^b	10.56 ± 0.75 ^b	8.75 ± 0.41 ^b	9.83 ± 0.73 ^b
Heart							
SOD	16.44 ± 0.83	14.53 ± 0.94 ^d	15.12 ± 0.72	16.58 ± 0.55 ^b	22.58 ± 0.91 ^b	28.43 ± 0.69 ^b	16.91 ± 0.68 ^b
CAT	2.25 ± 0.32	1.55 ± 0.26 ^d	1.58 ± 0.21	1.65 ± 0.21	1.86 ± 0.19 ^b	1.91 ± 0.20 ^b	1.65 ± 0.27
GSH-Px	9.91 ± 1.22	8.84 ± 0.53 ^d	9.54 ± 0.23 ^b	10.38 ± 0.25 ^b	10.97 ± 0.51 ^b	11.93 ± 0.48 ^b	10.52 ± 0.93 ^b
TAOC	0.98 ± 0.17	0.71 ± 0.09 ^d	0.85 ± 0.11 ^b	0.97 ± 0.08 ^b	1.31 ± 0.13 ^b	1.77 ± 0.08 ^b	1.06 ± 0.12 ^b
MDA	3.97 ± 0.29	5.13 ± 0.30 ^d	4.67 ± 0.17 ^b	3.96 ± 0.21 ^b	3.57 ± 0.24 ^b	2.95 ± 0.12 ^b	4.69 ± 0.12 ^b
Brain							
SOD	19.11 ± 0.97	17.8 ± 0.51 ^d	18.54 ± 1.05	20.58 ± 1.32 ^b	23.89 ± 1.35 ^b	27.82 ± 1.12 ^b	20.63 ± 0.86 ^b
CAT	4.41 ± 0.37	4.12 ± 0.24	4.23 ± 0.45	4.95 ± 0.11 ^b	5.21 ± 0.27 ^b	5.76 ± 0.31 ^b	4.31 ± 0.09 ^b
GSH-Px	4.30 ± 0.50	2.95 ± 0.43 ^d	3.35 ± 0.32	4.41 ± 0.52 ^b	6.03 ± 0.75 ^b	8.16 ± 0.48 ^b	5.73 ± 0.33 ^b
TAOC	0.92 ± 0.23	0.70 ± 0.08 ^d	0.76 ± 0.17	1.84 ± 0.37 ^b	2.33 ± 0.11 ^b	2.89 ± 0.19 ^b	2.04 ± 0.09 ^b
MDA	9.74 ± 1.38	11.28 ± 1.23 ^c	10.71 ± 0.88	8.27 ± 1.51 ^b	6.56 ± 0.85 ^b	4.27 ± 0.16 ^b	7.95 ± 0.63 ^b
Serum							
MDA	17.34 ± 2.12	32.49 ± 2.97 ^d	28.78 ± 2.04 ^b	23.83 ± 1.77 ^b	19.56 ± 1.83 ^b	16.63 ± 1.55 ^b	24.82 ± 2.79 ^b

The data were presented as means ± S.D. ($n = 10$) and evaluated by one-way ANOVA followed by the Student's *t*-test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$.

^a $P < 0.05$, compared with aged control group (II).

^b $P < 0.01$, compared with aged control group (II).

^c $P < 0.05$ compared with normal group (I).

^d $P < 0.01$ compared with normal group (I).

Table 2
Effect of polysaccharides on thymus and spleen index in aged mice

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Thymus index	0.385 ± 0.034	0.263 ± 0.027 ^c	0.273 ± 0.025	0.336 ± 0.040 ^b	0.457 ± 0.043 ^b	0.572 ± 0.063 ^b	0.431 ± 0.034 ^b
Spleen index	0.533 ± 0.052	0.452 ± 0.044 ^c	0.486 ± 0.082	0.512 ± 0.068 ^a	0.550 ± 0.080 ^b	0.591 ± 0.076 ^b	0.512 ± 0.042 ^b

The data were presented as means ± S.D. ($n = 10$) and evaluated by one-way ANOVA followed by the Student's *t*-test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$. Thymus or spleen index = weight of thymus or spleen/body weight × 100.

^a $P < 0.05$, compared with aged control group (II).

^b $P < 0.01$, compared with aged control group (II).

^c $P < 0.01$, compared with normal group (I).

3.4. Effect of the *Lycium barbarum* polysaccharides on antioxidant enzymes activity in brains in aged mice

Data on age-induced changes in brains are summarised in Table 1; there was only a slight change in CAT activity ($P > 0.05$), but significant decreases in SOD, GSH-Px activity, and TAOC, and an increase in MDA level with age compared with control young animals ($P < 0.05$, $P < 0.01$). Administration of polysaccharides and vitamin C dose-dependently elevated these antioxidant enzymes activity and reduced MDA level in brains (groups III–VII). Antioxidant activity of the polysaccharides in vivo was better than vitamin C at 500 mg/kg b.w.

3.5. Effect of the *Lycium barbarum* polysaccharides on thymus and spleen index in aged mice

As was shown in Table 2, significantly decreased thymus and spleen weight were observed with age ($P < 0.01$) in comparison with normal control. Administration of polysaccharides and vitamin C are seen to have remarkable effects on increasing the two indices in immune organ in aged mice in a dose-dependent manner (groups III–VII). The reversal of age-induced decreased thymus and spleen weight by polysaccharides administration is stronger than that by vitamin C at identical dose of 500 mg/kg b.w.

3.6. Effect of the *Lycium barbarum* polysaccharides on macrophage function in aged mice

Likewise, in the present study we observed that the tested indices (phagocytic index and phagocytic activity) markedly decreased with age (Table 3) ($P < 0.01$) in comparison with normal control. Supplementation of polysaccharides and vitamin C

both significantly raised the two indices in a dose-independent manner in aged mice (groups III–VII). In terms of the effect on phagocytic indices, polysaccharides administration was basically consistent with vitamin C of identical given dose but stronger than the latter on stimulating phagocytic activity.

3.7. Effect of the *Lycium barbarum* polysaccharides on LPF level in tested organs in aged mice

Table 4 represents the effect of polysaccharides and vitamin C on the levels of LPF in different tested organs in control and experimental animals. The LPF level in aged mice is markedly higher than that in young mice ($P < 0.01$). A significant reduction ($P < 0.01$) was found in the levels of LPF in all experiment animals groups (groups V–VII), when compared with aged control.

Moreover, the level was dose-independently decreased in polysaccharides-treated animals (groups III–VI). It was found in the present study that polysaccharides administration was still more effective in reducing LPF level than vitamin C at the identical dose.

3.8. Effect of the *Lycium barbarum* polysaccharides on serum MDA level in aged mice

Effect of the *Lycium barbarum* polysaccharides on serum MDA level in aged mice was shown in Table 1. Significant differences in serum MDA level were detected between normal control and aged control groups ($P < 0.05$). Data from polysaccharides treatment were pooled and compared to the aged control. Result indicated that polysaccharides treatment significantly decreased serum MDA level ($P < 0.05$) compared with aged control.

Table 3
Effect of polysaccharides on macrophage function in aged mice

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Phagocytic index (<i>k</i>)	3.32 ± 0.44	1.93 ± 0.25 ^d	2.17 ± 0.22	2.65 ± 0.43 ^b	2.87 ± 0.58 ^b	3.12 ± 0.45 ^b	3.18 ± 0.33 ^b
Phagocytic activity (α)	87.31 ± 5.65	72.06 ± 2.98 ^c	73.83 ± 4.32	79.97 ± 7.11	81.42 ± 7.38 ^a	86.43 ± 8.39 ^a	76.51 ± 7.39

The data were presented as means ± S.D. ($n = 10$) and evaluated by one-way ANOVA followed by the Student's *t*-test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$. Phagocytic activity, expressed by the percentage of phagocytosed neutrophil in 100 cells and phagocytic index, determined on the unit of Staphylococci ingested by single PMNs, was counted in 100 cells.

^a $P < 0.05$, compared with aged control group (II).

^b $P < 0.01$, compared with aged control group (II).

^c $P < 0.05$, compared with normal group (I).

^d $P < 0.01$, compared with normal group (I).

Table 4
Effect of polysaccharides on LPF level ($\mu\text{g/g}$ tissue) in tested organs in aged mice

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Brain	0.224 \pm 0.027	0.440 \pm 0.041 ^b	0.434 \pm 0.036	0.358 \pm 0.051 ^a	0.312 \pm 0.031 ^a	0.285 \pm 0.027 ^a	0.398 \pm 0.022 ^a
Heart	0.115 \pm 0.009	0.187 \pm 0.014 ^b	0.180 \pm 0.024	0.174 \pm 0.011 ^a	0.160 \pm 0.012 ^a	0.133 \pm 0.013 ^a	0.184 \pm 0.023
Liver	0.245 \pm 0.019	0.444 \pm 0.036 ^b	0.360 \pm 0.019 ^a	0.351 \pm 0.014 ^a	0.330 \pm 0.011 ^a	0.284 \pm 0.027 ^a	0.348 \pm 0.019 ^a
Kidney	0.206 \pm 0.011	0.382 \pm 0.023 ^b	0.371 \pm 0.025	0.344 \pm 0.019 ^a	0.305 \pm 0.019 ^a	0.284 \pm 0.013 ^a	0.369 \pm 0.024

The data were presented as means \pm S.D. ($n = 10$) and evaluated by one-way ANOVA followed by the Student's t -test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$.

^a $P < 0.01$, compared with aged control group (II).

^b $P < 0.01$, compared with normal group (I).

Moreover, the compound of polysaccharides plus vitamin C exhibited stronger antioxidant activity than either of the two antioxidants.

4. Discussion

Aging is a progressive deterioration of physiological function that impairs the ability of an organism to maintain homeostasis and consequently increases the organism's susceptibility to disease and death (Nohl and Hegner, 1978). Nearly all organisms manifest functional declines as a result of aging. It is widely accepted that disorganizing free radical reactions linked to oxygen metabolism or "oxidative stress" (Chance et al., 1979; Sies, 1986; Gutteridge, 1987) play an important role not only in normal aging (Harman, 1956; Lesser, 2006) but also in many age-related degenerative processes (Harman, 1981).

Oxidation of lipids produces lipid peroxides that can reduce membrane fluidity, inactivate membrane-bound proteins and decompose into cytotoxic aldehydes such as malondialdehyde or hydroxynonenal (Richter, 1987). Accumulation of hydroxynonenal increases with age in several *Drosophila* tissues (Zhang and Xu, 2006) and the level of malondialdehyde and hydroxynonenal-conjugated collagen protein increases with age in rat tissue (Noberasco et al., 1991). We have also observed an increase in the levels of MDA, a marker of lipid peroxidation in the test organs of aged mice. Hence, lipid oxidation is closely associated to aging. On the contrary, LBP treatment demonstrated decreased level of lipid peroxides and this could be in part due to reduced formation of lipid peroxides from age-dependent free radicals.

A vast number of evidence implicates that aging is associated with a decrease in antioxidant status and that age-dependent increases in lipid peroxidation are a consequence of diminished antioxidant protection (Schuessel et al., 2006; Alvarado et al., 2006), being in agreement with our current study. The major antioxidant enzymes, including SOD, GPX and CAT, are regarded as the first line of the antioxidant defense system against reactive oxygen species generated in vivo during oxidative stress. SOD dismutates superoxide radicals to form hydrogen peroxide, which in turn is decomposed to water and oxygen by GSH-Px and CAT, thereby preventing the formation of hydroxyl radicals (Yao et al., 2005). Therefore, these enzymes act cooperatively at different sites in the metabolic pathway of free radicals. However, there have been many conflicting studies concerning age-related changes of the antioxidant defenses

in various organs. For example, some previous reports (Yüksel and Asma, 2006; Miquel et al., 2006) have shown that some of the antioxidant enzymes in important organs such as liver, heart, kidney, and brain, were decreased with aging, whereas, other investigators have indicated no alteration or increased activities in the antioxidant enzymes (Rolo and Palmeira, 2006; Masztalerz et al., 2006). The differences among those data might be, in part, due to differences in the animal's sex, strain, and age used, assay method, the enzyme property examined, and/or experimental conditions used. In our study along with increased lipid peroxidation, age-induced oxidative injury was found to reduce the total antioxidant capacity (TAOC), which reflects the non-enzymatic antioxidant defense system, as well as antioxidant enzyme levels (SOD, CAT, GSH-Px) in test organs of aged mice and this observation concurs with earlier findings (Kogan et al., 2005). Due to depletion in antioxidant levels, the free radicals are not neutralized and aged organs show enhanced susceptibility to lipid peroxidation. The observation that LBP treatment significantly restores the marker enzymes activity of aged mice compared to aged control suggests the reversal by these drugs administration of age-induced oxidation. The enhanced activity of SOD, CAT and GSH-Px and increased TAOC in the aging animals can be very effective in scavenging the various types of oxygen free radicals and their products. So the inhibitory effect of the *Lycium barbarum* polysaccharides on lipid peroxidation might be, at least in part, attributed to its influence on the antioxidant enzymes and non-enzymatic system. Govind et al. (1990) have reported that, in general, the age-related changes in the activities of SOD, GSH-Px, and CAT were paralleled by a similar change in the relative level of the mRNA expressions coding for these enzymes in brain, hepatocytes, and kidney. As for lung, Gomi and Matsuo (2002) have shown that aging decreased the mRNA expressions of SOD and GSH-Px but did not change CAT. Their study also revealed the discrepancy between the activity and mRNA expression of either SOD or GSH-Px. Thus, these findings, including the current findings, may suggest that the activities of antioxidant enzymes in aged tissues could be controlled by translational process and/or post-translational process, but not by transcriptional process. Future investigation, however, will be required to determine additional mechanisms. It is possible that the effect of the *Lycium barbarum* polysaccharides on SOD, CAT and GSH-Px was associated with its effect on translational process and/or post-translational process of these antioxidant enzymes.

A variety of immune changes occur in both animals and humans with increasing age. The aging of the immune system (immunosenescence) is associated with dramatic reductions in immune responsiveness as well as functional dysregulation (Burns, 2004). This translates into less effective innate and adaptive immune responses, increased reactivity against self-antigens (autoimmunity) and decreased incidences of infectious diseases and cancer (Hale et al., 2002). As shown in Tables 2 and 3, the decreased thymus and spleen indices, and macrophage function in aged mice was a good indicator of age-induced decline in immune function. The administration of the *Lycium barbarum* polysaccharides enhance spleen index, thymus index, phagocytic index, phagocytic activity in aging mice, suggesting that the *Lycium barbarum* polysaccharides effectively stimulated the immune mechanism of aged mice. Although the exact mechanism on the immune-stimulating activity of the *Lycium barbarum* polysaccharide is not known, it has been proposed that it may act by inducing a number of antioxidant enzymes (SOD, CAT, GSH-Px) and decreasing lipid peroxidation due to its stimulating translational process and/or post-translational process of these antioxidant enzymes.

At last, the influence of the *Lycium barbarum* polysaccharides on the LPF in aged mice was also investigated. LPF is an important marker for oxidative damage. The deposition of LPF in animal tissues usually increased significantly with age (Philipp et al., 2005). Large deposits of the pigment, which can contain toxic compounds such as the amphiphilic pyridinium bisretinoid, A2E, may be deleterious to cell function and contribute to disease progression (Terman, 2001). In the present study, the obtained data showed that its content increased continuously with age in mice, as found in several other species (Leeuwenburgh et al., 1994; Zielinski and Pörtner, 2000; Bluhm et al., 2001; Sukhotin et al., 2002). Therefore, our result confirms age-dependent accumulation of LPF. An alternative possibility is that lipofuscin is continually turned-over in tissues, the characteristic age-related accumulation being due to imbalance between rate of formation and rate of degradation or elimination, with more being produced than is removed. Likewise, the *Lycium barbarum* polysaccharides effectively reduced its content in aged tissues. We assume that the inhibition of the *Lycium barbarum* polysaccharides against the LPF deposition is indirectly achieved by its scavenging free radical and stimulating antioxidant enzymes activity. Moreover, addition of vitamin C to polysaccharides can significantly and dose-dependently raise antioxidant activity of polysaccharides, i.e. there is a noticeable cooperative effect between them, which is well reflected in elevation of CAT, SOD, GSH-Px activity, TAOC, and reduction of MDA, LPF level in tested organs of aged mice, and improvement of immunity function (Tables 1–4).

The findings of our study have clearly indicated that aged animals are subjected to a more intense oxidative environment than young animals and have impaired antioxidant status. In the present study, we have administered antioxidants (LBP, vitamin C) to aged mice and have demonstrated its beneficial effects in terms of effectively mitigating oxidative stress through effectively attenuating lipid peroxidation as a biomarker of oxidative stress and enhancing antioxidant enzymes activi-

ties and stimulating the immune system in vivo. Therefore, LBP administration is a useful and an attractive proposition for counteracting oxidative stress and various other detrimental factors associated with aging.

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