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Health-promoting effect of LBP and healthy Qigong exercise on physiological functions in old subjects

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ABSTRACT

The aim of this study was to ascertain whether the *Lycium barbarum* polysaccharides (LBP) and healthy Qigong exercise had an effect on the prevention of atherosclerosis in the elderly. LBP was prepared and determined using High-performance liquid chromatography (HPLC). Results showed that the LBP mainly consisted of rhamnose, arabinose, ribose, mannose, galactose, fructose, xylose and glucose with a molar ratio of 1:6.78:0.21:0.63:9.34:0.51:3.11:2.82. LBP was administrated to male subjects for 3 months. Pharmacological function of LBP was further compared with Healthy Qigong exercise. On the basis of experimental result, LBP administration and healthy Qigong exercise is believed to aid in preventing atherosclerosis by the modulation of blood pressure, and by reducing plasma triglycerides, total cholesterol, and low density lipoprotein (LDL) cholesterol, elevating HDL cholesterol in the elderly. The health-promotion effect healthy Qigong exercise can be comparable to that of LBP.

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1. Introduction

With the population increasing in age, an increase in the prevalence of age-related disorders can be anticipated. A common observation in aging is an increase in cognitive functions (Rabbit & Lowe, 2000). Several cardiovascular risk factors, including high blood pressure (BP) might play an important role in this cognitive deterioration. The prevalence of high BP is known to increase with aging (Efstratopoulos et al., 2006). High BP furthermore, both at middle and old age, has been associated with a decline in cognitive ability. Another common observation in aging is a increase in coronary heart disease. Coronary heart disease remains the leading cause of death in the elderly population (Ades, Waldmann, Polk, & Coflesky, 1992). One of the major risk factors is hypertension. Hypertension is an important risk factor for cardiovascular mortality and morbidity in epidemiologic studies. Improvements in identification and treatment of hypertension have contributed to a major reduction in the incidence of cardiovascular disease in many countries (Gayda, Temfemo, Choquet, & Ahmaïdi, 2004).

Healthy Qigong is an ancient form of exercise with origins in traditional Chinese martial and healing arts. It is widely practiced in Asia, particularly among the elderly, and has gained increasing popularity in the United States. Recent literature has suggested that it may be particularly suitable for the elderly or de-conditioned patient with cardiac disease. Healthy Qigong incorporates slow-moving, gentle physiological activity, balance, and weight shifting, with meditation, relaxation, deep breathing, and imagery. Reported benefits of Healthy Qigong include increased balance and decreased incidence of falls, increased strength and flexibility, reduced pain and anxiety, improved self-efficacy, improved sleep and enhanced cardiopulmonary function (Yeh et al., 2004). An earlier systematic review has reported limited evidence of its effects in preventing falls in view of paucity of randomized controlled trials. There has been recent extensive research on the effects of Healthy Qigong in fall prevention among the elderly. Improved exercise capacity and quality of life have been reported in patients with heart failure (McKelvie et al., 1995).

Fruit from *L. barbarum* L. in the family Solanaceae is well-known in traditional Chinese herbal medicine and nowadays has been widely used as a popular functional food, with a large variety of beneficial effects, such as reducing blood glucose and serum lipids, anti-aging, immuno-modulating, anticancer, anti-fatigue, and male fertility-facilitating (Chen, Zhong, Zeng, & Ge, 2008; Gao, Xu, & Li, 2000; Niu, Wu, Yu, & Wang, 2008; Peng, Huang, Qi, Zhang, & Tian, 2001; Peng, Qi, Tian, & Zhang, 2001). In vivo, LBP could dose-dependently decrease tumor weight and increase the amount of splenocytes, proliferation of activated T cells, NK activity and

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TNF- α levels in S180-bearing mice (Zhang et al., 2005). In vitro, 20–1000 mg/L LBP could inhibit the growth of human leukemia HL-60 cells in dose-dependent manner and decrease the membrane fluidity (Ebringerová et al., 2008). Some constituents of *L. barbarum* fruits have been chemically investigated, especially *L. barbarum* polysaccharide (LBP) components. Five polysaccharides (glycoconjugates) (LbGp1–LbGp5) were isolated and structurally elucidated (Gao et al., 2000; Peng, Huang, et al., 2001). Basing on some literatures, we can assume that extract of *L. barbarum* should be attributed to its polysaccharides.

The objectives of this study are to evaluate the effects of LBP on physiological index by comparing its health-promoting effects with Healthy Qigong in old people.

2. Subjects and methods

2.1. Materials

Dried *L. barbarum* fruits were purchased from the local market (Shanghai city, China).

2.2. Preparation of polysaccharides

Polysaccharides from L. barbarum were prepared by the method of Yin and Dang (2008). The dried fruit samples (10 g) were ground to fine powder and put in given volume (water/raw material, v/v, 20-50) of hot water (30, 35, 40, 45, 50, 55 and 60 °C) and the extraction was carried out during different periods (1, 1.5, 2, 2.5, 3, 3.5, and 4 h). The extract 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 several times (1-5) in given volume (water/raw material, v/v, 20-50) of hot water (30, 35, 40, 45, 50, 55 and 60 °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.

The content of the polysaccharides was measured by phenolsulfuric method (Masuko et al., 2005).

2.3. HPLC analysis

Chromatographic analyses were performed on a Waters 600 HPLC system equipped with a Waters 996 photodiode array spectrophotometer, a 7125 Rheodyne injector (with 20, 50, 100 or 200 μ L loop), and a Waters in-line degasser apparatus. Chromatographic data were collected and processed on a computer running with Empower software from Waters (Milford, MA, USA).

Chromatography was performed at 150 °C (otherwise, specified). The column void time (t_0) was considered to be equal to the peak of the solvent front and was taken from each particular run. The mobile phase was acetonitrile/water (20:80, v/v) at a flow rate of 0.4 ml/min. In the postcolumn procedure, 250 mM hydrochloric acid (HCl) was added to the column eluate at a flow rate of 0.1 ml/min and mixed to form the hydrolytic reaction mixture, which was passed through a polytetrafluoroethylene (PTFE) tube (0.5 mm i.d., 5 m) thermostated at 150 °C. Subsequently, a 450mM NaOH solution containing 110 mM guanidine was added to the hydrolytic reaction mixture at a flow rate of 0.1 ml/min and mixed to form the fluorometric reaction mixture, which was passed through a stainless-steel tube (0.5 mm i.d., 10 m) thermostated at 90 °C. Finally, the mixture was cooled down in another stainless-steel tube (0.25 mm i.d., 3 m) at ambient temperature and detected.

2.4. Subjects

The study included 177 healthy free-living subjects aged between 52 and 73 years (75 men and 102 women). Subjects were recruited from YangPu district in shanghai city in china. All subjects were in good health condition, and without functional impairment. Healthy status was evaluated by a specific questionnaire on health and morbidity planned for the study and filled in by the general practitioner after medical examination and analysis of the clinical record. None of the subjects studied was taking any drug that may interfere with test index, particularly nutritional integrators or antioxidant supplements.

2.5. Experiment design

All male subjects were classified in six groups: Normal group (M), Polysaccharides-treated group (M), Healthy Qigong exercise group (M). Normal group (M) was further classified into two subgroups Ia and IIa; Polysaccharides-treated group (M) was further classified into two subgroups IIIa and IVa; Healthy Qigong exercise group (M) was further classified into two subgroups Va and VIa. All female subjects were classified in six groups: Normal group (F), Polysaccharides-treated group (F), Healthy Qigong exercise group (F). Normal group (F) was further classified into two subgroups Ib and IIb; Polysaccharides-treated group (F) was further classified into two subgroups IIIb and IVb; Healthy Qigong exercise group (F) was further classified into two subgroups Vb and VIb.

In all the participants, clinical laboratory analyses were determined, including measurements of Systolic pressure, Diastolic pressure, Blood pressure drop, total cholesterol, triglycerides, HDL-c, LDL-c, HDL/LDL. All subjects gave informed consent to the study.

All above-mentioned indexes in subjects in subgroups Ia and b, Illa and b, Va and b (as control) were first measured before experiment. Subjects in subgroups IIa and b were not given any treatment. Subjects in subgroups IVa and b were orally given LBP at daily dose of 100 mg/kg BW for 3 months. Subjects in subgroups VIa and b regularly performed Healthy Qigong exercise for 3 months. After 3 months experiment, all above-mentioned indexes in subgroups IIa and b, IVa and b, VIa and b were measured.

2.6. Biochemical analysis

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a Dinamap (Crikton, Inc.) system according to China Heart Association recommendations. Blood pressure (BP) drops were calculated according to the method described by Efstratopoulos et al. (2006).

TC, TG, HDL-c, LDL-c were determined by using assay kit (Caymen Chemical, USA). All the procedures were performed following the manufacturer's instruction manual.

2.7. Statistical analysis

Statistical analysis was performed with the program Statistical Package for the Social Sciences (SPSS version 12.0, Chicago, IL) for Windows. All data are presented as means \pm SD. All tests were two-tailed and considered significant at P < .05.

3. Results

3.1. Chemical components of polysaccharides

Compared with standard saccharides (Fig. 1) (rhamnose, arabinose, xylose, mannose, glucose and galactose), the HPLC results indicated that the LBP mainly consisted of rhamnose, arabinose,

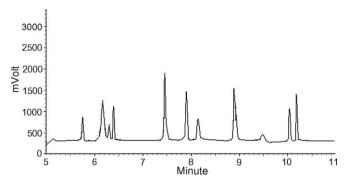


Fig. 1. HPLC of standard sample.

ribose, mannose, galactose, fructose, xylose and glucose (Fig. 2). These sugars (rhamnose, arabinose, ribose, mannose, galactose, fructose, xylose and glucose) were found to be present in a molar ratio of 1:6.78:0.21:0.63:9.34:0.51:3.11:2.82. The sugar composition of the LBP was consistent with the results of Tian and Wang's work (2006), except that ribose and fructose weren't found in the latter.

3.2. Blood pressure in old men

The blood pressures in men are given in Table 1. Compared with the control (group IIIa), The LBP administration significantly increased or decreased SBP, DBP and BP drop value in group IVa (polysaccharides-treated group) (P < .05, P < .01). Compared with the control (group Va), the Healthy Qigong exercise significantly increased or decreased SBP, DBP and BP drop value in group VIa (Healthy Qigong exercise group) (P < .05, P < .01). However, there were no significant differences (P > .05) in SBP, DBP and BP drop between the normal groups (Ia and IIa).

3.3. Blood pressure in old women

The blood pressures in women are given in Table 2. Compared with the control (group IIIb), The LBP administration significantly increased or decreased SBP, DBP and BP drop value in group IVb (polysaccharides-treated group) (P < .05, P < .01). Compared with the control (group Vb), the Healthy Qigong exercise significantly increased or decreased SBP, DBP and BP drop value in group VIb (Healthy Qigong exercise group) (P < .05, P < .01). However, there were no significant differences (P > .05) in SBP, DBP and BP drop between the normal groups (Ib and IIb).

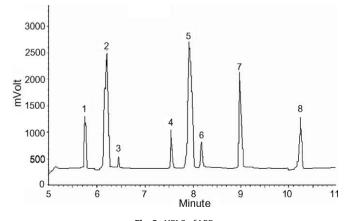


Fig. 2. HPLC of LBP.

3.4. Blood-lipid in old men

In this study, we investigated the effects of LBP and Healthy Qigong exercise on levels of plasma TC, TG, HDL-c and LDL-c from 120 male subjects (Table 3). It can be observed that TC, TG, LDL-c and HDL-c levels in blood from the male subjects fed the LBP (group IVa) were significantly reduced or enhanced (P < .05, P < .01) compared to the control (IIIa). We also found that Healthy Qigong exercise could significantly reduce TC, TG, LDL-c levels and enhance HDL-c level in blood of the male subjects (group VIa) compared to the control (Va). Abnormal levels of TC and LDL-c could be found in group IIa. However, there were no significant differences (P > .05) in TG, and HDL-c between the normal groups (Ia and IIa).

3.5. Blood-lipid in old women

As shown in Table 4, the LBP administration could significantly decrease plasma TC, TG, LDL-c and enhance HDL-c levels (P < .05, P < .01) from 40 female subjects in group IIIb compared with the control group (IVb). In addition, we also observed that the Healthy Qigong exercise could also significantly decrease plasma TC, TG, LDL-c and enhance HDL-c levels (P < .05, P < .01) from 40 female subjects in group Vb compared with the control group (VIb). Abnormal blood lipid indexes were observed between the normal groups (Ib and IIb).

4. Discussion

In modern societies, blood pressure rises with age. High blood pressure, or hypertension, commonly has no symptoms, and most people don't even know that they have it until it has damaged their heart or brain. It is aptly named "the silent killer". Treating hypertension helps prevent stroke and coronary heart disease in middle aged and elderly people younger than 80. High blood pressure significantly increases your risk for getting heart disease and/or kidnev disease, and for having a stroke. While there may be no symptoms, and people affected by high blood pressure may feel fine, some may experience dizziness, palpitations, sweating, and headaches. Blood pressure is typically recorded as two numbers - the systolic pressure (as the heart beats) over the diastolic pressure (as the heart relaxes between beats). Usually both the systolic and the diastolic pressures are elevated. However, in the elderly, only the systolic pressure may be elevated, which nevertheless can cause medical problems down the road.

In elderly healthy subjects, LBP consumption enhanced systolic blood pressure and blood pressure drop, reduced diastolic blood pressure, normalizing these values to those of normotensives. These variance in the levels of blood pressure after LBP consumption are associated with improved heart function (Taylor-Piliae & Froelicher, 2004; Vetvicka et al., 2007). Decreased capacity to use fat as fuel may theoretically be an important factor in older individuals because they have a smaller skeletal muscle mass, which is the primary site for fat oxidation (Melanson, Saltzman, Russel, & Roberts, 1997; Poehlman et al., 1992). Previous studies have documented several changes in the metabolism of lipids in the elderly population, which include a lesser lipolytic capacity of endogenous fat stores and a lesser capacity to oxidize fat (Melanson et al., 1997; Miranda et al., 2008: Toth & Tchernof, 2000). Chylomicron clearance, both lipolysis and hepatic uptake, is sensitive to LPL activity (Crawforda & Borensztajn, 1999; Wang & Luo, 2007). Thus, our finding of decreased LPL activity may slow chylomicron clearance in both stages and, as expected, was associated with enrichment of chylomicron particles with triglycerides. There are several key steps in chylomicron production, lipolysis and particle clearance where drugs may conceivably operate. These lipids must first be assimilated by enterocytes and this requires the breakdown of tri-

Table 1Blood pressure in men (Ding-hai Yu, Jing-mei Wu, Ai-jun Niu; carbohydrate polymers)

Index	Normal group		Polysaccharides-treated group		Shadowboxing group	
	0 month (Ia)	6 month (IIa)	0 month (IIIa)	6 month (IVa)	0 month (Va)	6 month (VIa)
SBP (mmHg)	130.00 ± 16.70	131.04 ± 14.83	133.21 ± 12.72	145.09 ± 13.72**	132.02 ± 17.33	146.99 ± 18.04
DBP (mmHg)	80.18 ± 12.21	79.15 ± 15.38	82.32 ± 12.94	78.28 ± 15.04	81.94 ± 13.44	77.22 ± 11.12°
BP drop (mmHg)	49.81 ± 10.42	51.89 ± 13.31	50.89 ± 14.11	66.81 ± 14.53**	50.11±13.47	69.77±10.77**

Indexes tested in 0 month vs those tested in 6 month.

Table 2Blood pressure in women (Ding-hai Yu, Jing-mei Wu, Ai-jun Niu; carbohydrate polymers)

Index	Normal group		Polysaccharides-treated group		Shadowboxing group	
	0 month (Ib)	6 month (IIb)	0 month (IIIb)	6 month (IVb)	0 month (Vb)	6 month (VIb)
SBP (mmHg)	128.94 ± 17.31	127.85 ± 20.46	128.08 ± 18.54	136.47 ± 10.25**	127.84 ± 13.05	143.03 ± 18.91**
DBP (mmHg)	80.02 ± 11.73	81.97 ± 12.56	81.13 ± 15.16	71.33 ± 6.52**	80.54 ± 12.03	75.24 ± 12.27°
BP drop (mmHg)	48.92 ± 10.04	45.88 ± 14.69	46.95 ± 13.31	65.14 ± 9.22**	47.30 ± 14.31	67.79 ± 13.74**

Indexes tested in 0 month vs those tested in 6 month.

Table 3 Blood-lipid in men (Ding-hai Yu, Jing-mei Wu, Ai-jun Niu; carbohydrate polymers)

Index	Normal group		Polysaccharides-treated group		Shadowboxing group	
	0 month (Ia)	6 month (IIa)	0 month (IIIa)	6 month (IVa)	0 month (Va)	6 month (VIa)
TC (mmo/l)	4.37 ± 0.31	4.90 ± 0.19 [*]	4.73 ± 0.32	4.62 ± 0.45**	4.62 ± 0.69	4.31 ± 0.41
TG (mmo/l)	1.42 ± 0.86	1.44 ± 0.25	1.41 ± 0.23	1.07 ± 0.21**	0.98 ± 0.12	0.69 ± 0.06
HDL-c (mmo/l)	1.12 ± 0.24	1.14 ± 0.20	1.10 ± 0.20	1.58 ± 0.25	1.14 ± 0.24	1.33 ± 0.19°
LDL-c (mmo/l)	2.69 ± 0.27	2.81 ± 0.22	2.61 ± 0.39	2.46 ± 0.44	2.79 ± 0.29	2.43 ± 0.13 **

Indexes tested in 0 month vs those tested in 6 month.

Table 4 Blood-lipid in women (Ding-hai Yu, Jing-mei Wu, Ai-jun Niu; carbohydrate polymers)

Index	Normal group		Polysaccharides-treated group		Shadowboxing group	
	0 month (Ib)	6 month (IIb)	0 month (IIIb)	6 month (IVb)	0 month (Vb)	6 month (VIb)
TC (mmo/l)	4.86 ± 1.02	5.42 ± 1.12 [*]	4.81 ± 0.94	4.75 ± 0.83°	5.05 ± 0.92	4.98 ± 0.8
TG (mmo/l)	1.40 ± 0.72	1.67 ± 1.29	1.48 ± 0.77	1.24 ± 0.68	1.75 ± 1.27	1.19 ± 0.52**
HDL-c (mmo/l)	1.22 ± 0.27	1.28 ± 0.22	1.19 ± 0.18	1.53 ± 0.21	1.33 ± 0.38	1.38 ± 0.37
LDL-c (mmo/l)	2.92 ± 0.82	3.31 ± 0.85**	2.82 ± 0.68	2.57 ± 0.39°	2.77 ± 0.70	$3.04 \pm 0.68^{\circ}$

Indexes tested in 0 month vs those tested in 6 month.

acylglycerol to fatty acids and partial acylglycerols by the action of pancreatic lipase and the release of cholesterol from its ester by an esterase. In this study, LBP consumption induces significant changes in the levels of plasma lipid of experimental subjects. In both cases (male subjects and female subjects), TC, TG and LDL levels significantly decreased in elderly humans after long-term LBP consumption. In addition, HDL levels significantly increased in elderly humans after long-term LBP consumption. In our study, statistical analysis revealed that decreased plasma LDL was associated with increased HDL cholesterol, a profile that is associated with increased risk for atherosclerosis (Gayda et al., 2004; Zhao et al., 2007). Thus, our findings support a novel property of LBP in protecting against coronary heart disease in elderly population.

As compared with high-impact exercise, low-weight-bearing Healthy Qigong exercise is a unique form of physiological activity that involves neuromuscular coordination, low velocity of muscle

contraction, and low impact; it involves no jumping, and is recommended for elderly people to practice regularly, even for the elderly with osteoporotic and other cardiovascular chronic conditions (Equiluz-Bruck, Schnack, Kopp, & Schernthaner, 1996; Song, Lee, Lam, & Bae, 2003; Sun, Pu, Huang, & He, 2000). In the present study, the Healthy Qigong exercise groups showed significantly better physiological indexes, in terms of blood pressure and plasma lipid. Available studies of LBP groups showed that regular LBP administration had also improved or maintained health status by improving plasma lipid, and thereby reducing blood lipid among older individuals (Zhu & Zhang, 2005). This was supported by our work. Therefore, LBP and healthy Qigong exercise should have beneficial effects not only in retarding age-related decline of physiological functions but also in decreasing the risks of cardiovascular diseases. The study had suggested that healthy Qigong exercise has good effects on plasma lipid concentrations, which

^{*} P < .05.

^{**} P < .01.

^{*} P < .05.

[&]quot; P < .01.

^{...} P < .05.

^{**} P < .01.

^{*} P < .05.

^{**} P < .01.

can be comparable to that of LBP. This can be mainly attributed to the health-promotion effect of healthy Qigong exercise.

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