A STUDY OF LYCIUM BARBARUM POLYSACCHARIDES (LBP) EXTRACTION TECHNOLOGY AND ITS ANTI-AGING EFFECT

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Abstract

The objective of the study was to optimise the LBP extraction technology and to study the anti-aging effect of LBP by establishing D-gal aging mouse model. Orthogonal design was used to study the extraction technology The experimental aging mouse model was formed by continuous injection of D-gal, and the anti-aging capacity of LBP was tested using measuring MDA, CAT and GSH-px contents and SOD activity in blood and SOD, MDA and Hyp levels in skin. The results showed that the optimum LBP extraction option determined by the orthogonal design is as follows: solid-liquid ratio of 1:30, extraction for 2 times, 90 min each time, and power is 100 kHz. Thus, LBP can increase SOD, CAT and GSH-px levels in blood and reduce MDA level. It can also improve skin SOD activity, reduce skin MDA content, and increase Hyp content. We concluded that the extraction method established in this experiment is easy and feasible, and the yield of LBP is high, apparently showing that LBP has the potential of delaying senility in D-gal induced mice.

Keyword: LBP; extraction; anti-aging; D-gal

Introduction

Fructus Lycii is ripe fruit of Lycium barbarum L, with the functions of nourishing liver and kidney and replenishing vital essence to improve eyesight. The main chemical constituents are LBP and betaine, and it is mainly used to enhance immunity (Xiao, 2010), delay senility (Raymond, 2008; Shi, 2008), reduce blood glucose (Tan, 2008) and promote the synthesis of nucleic acids and proteins in modern medicine. It also has anti-tumour (Fang, 2011; Xu, 2000), antioxidation (Li, 2006), anti-cardiovascular and other effects. Considering the edibility of LBP, this experiment selects water as the solvent for extraction.

Materials and Methods

Drug and reagents

Lycium (Ningxia Zhongning Qixiangyuan Trading Co., Ltd); phenol and concentrated sulfuric acid (analytical pure): D-gal (Beijing BioDee Biotechnology Co., Ltd); anhydrous glucose (Hebei Shengxue Glucose Co., Ltd); SOD, MDA, HYP and GSH-px kits (Nanjing Jiancheng Bioengineering Institute)

Animals

50 Wistar mice (Harbin Medical University Laboratory Animal Center)

Instruments

PT1600E tissue homogeniser (Zhejiang Machinery Factory); DL-4000B refrigerated centrifuge (Henan Kaida Scientific Instrument Co., Ltd.)

Standard curve drawing (Gong, 2005)

0.1005 g of reference anhydrous glucose, which was dried to constant weight at 105□, was precisely weighed and added into a 100ml volumetric flask. It was dissolved in distilled water and diluted to the scale. 1.0, 2.0, 4.0, 6.0, 8.0, 10.0ml of reference stock solutions were precisely measured respectively and added into 100ml volumetric flasks. They were diluted with water to the scale. Then, 1.0ml was taken out respectively and added into test tubes with stopper. 1ml of 5% phenol solution was added respectively. After shaking, 5.0ml of concentrated sulphuric acid was added. After immediate mixing, the tubes were placed in cold bath to room temperature. With distilled water as the blank control, the absorbance value was measured at 490nm. The standard curve was drawn with absorbance value as the horizontal axis and concentration as the vertical axis to obtain the linear equation Y=11.653X+0.0392, r=0.9996 (n=6).

LBP extraction and content measurement (Zhi, 2004)

According to the preliminary experiment, the main factors (solvent amount, extraction time, ultrasonic power and extraction times) affecting the ultrasonic water extraction technology are selected, and the $L_9 3^4$ orthogonal experiment is made on 4 factors and 3 levels. The levels of the factors are shown in Table 1.

Table 1: Levels of the factors

Level	Factor			
	A Solid-liquid ratio (times)	B Extraction time (min)	C Ultrasonic power (kHz)	D Extraction times (times)
1	10	30	60	1
2	20	60	80	2
3	30	90	100	3

Sample measurement method is same as 2.1, and LBP content is calculated according to the following formula:
LBP content (%) = (C×Df/m) × 100%
C=Glucose content in the sample solution μg/ml; D=Dilution times of the sample solution; f= conversion factor; m=Sample mass μg

Animal model making and grouping

60 Wistar mice with average weight of 25±1g, half males and half females, are randomly grouped, 10 in each group, that is, the normal group, the model aging group, the model low dose group, the model medium dose group, the model high dose group and the model vitamin E control group.

The normal group was administrated through subcutaneous injection of 100mg/kg·d saline and gastric infusion of 1ml/100g·d saline every day. The model aging group was given subcutaneous injection of 100mg/kg·d D-gal and gastric infusion of 1ml/100g·d saline every day. The model low dose group was given subcutaneous injection of 100mg/kg·d D-gal and gastric infusion of 10ml/100g·d LBP water solution every day. The model medium dose group was given subcutaneous injection of 100mg/kg·d D-gal and gastric infusion of 20ml/100g·d LBP water solution every day. The model high dose group was given subcutaneous injection of 100mg/kg·d D-gal and gastric infusion of 40ml/100g·d LBP water solution every day. And the model vitamin E control group was given subcutaneous injection of 100mg/kg·d D-gal and gastric infusion of 5mg/100g·d LBP vitamin E solution every day, for continuous 30 days, and subcutaneous injection and gastric infusion were operated under sterile conditions.

Indicator measurement

Blood was collected from orbits, and serum was separated for later use. The animals were sacrificed and the back skin with the hair was removed. Fat and connective tissues were removed, and the remaining mass was weighed, rinsed with ice-cold saline, placed in the homogeniser, and homogenised with 9 times of saline for 10min. It was prepared into 10% homogenate, followed by centrifugation at 4000r/min for 10min before the supernatant was collected for later use.

MDA content and CAT content in serum, GSH-px activity and SOD activity in whole blood, and MDA level, SOD level and Hyp level in skin were measured respectively in accordance with the requirements in the instructions.

Statistical processing

SPSS 13.0 statistical analysis software is used. The measured data are expressed with x±s, and intergroup comparison uses t test.

Results

LBP extraction and content measurement

Table 2: Orthogonal experiment results

Experiment No.	A Solid-liquid ratio (times)	B Extraction time (min)	C Ultrasonic power (kHz)	D Extraction times (times)	LBP content (%)
1	1	1	1	1	9.85
2	1	2	2	2	10.22
3	1	3	3	3	11.58
4	2	1	2	3	10.56
5	2	2	3	1	11.86
6	2	3	1	2	12.55
7	3	1	3	2	13.57
8	3	2	1	3	12.73
9	3	3	2	1	13.12
Mean1	10.550	11.327	11.710	11.610	
Mean 2	11.657	11.603	11.300	12.113	
Mean 3	13.140	12.417	12.337	11.623	
Range	2.590	1.090	1.037	0.503	

Table 3: Variance analysis results

Variance source	Sum of square of deviations	Degree of freedom	F ratio	P
A	10.133	2	20.512	<0.05
B	1.926	2	3.899	>0.05
C	1.635	2	3.310	>0.05
D	0.494	2	1.000	>0.05

P<0.05 means significant difference

The orthogonal experiment results are shown in Table 2, and the variance analysis is shown in Table 3. The intuitive analysis and variance analysis show that the sequence of the factors affecting LBP extraction technology is solid-liquid ratio, > extraction time > ultrasonic power > extraction times, in which solid-liquid ratio has significant effect (P<0.05) on the extraction technology. According to the experiment results, the optimum LBP extraction technology can be determined as A3B3C3D2, that is, solid-liquid ratio is 1:30, extraction for two times, 90min each time, and the power is 100 kHz.

Indicator measurement results

The effect of LBP on SOD, MDA, CAT and GSH-px activities in blood of aging model mouse

Compared with the normal group, SOD, CAT and GSH-px activities in the model aging group decrease. SOD, CAT and GSH-px activities in the drug use groups and the vitamin E group can be significantly increased, and as drug concentration increases, the activity increase is more significant. Compared with the normal group, MDA content in mouse blood in the model aging group increases, and MDA content in the drug use groups and the vitamin E group can be reduced, as shown in Table 4.

Table 4: The effect of LBP on SOD, MDA, CAT and GSH-px in mouse blood

Group	SOD（U/mg）	MD（nmol/mg）	CAT（U/mg）	GSH-px（mg/g）
Normal group	175.74±10.23	4.58±0.35	25.18±1.62	190.74±9.87
Aging group	142.55±8.43[1)]	6.41±0.62[1)]	18.64±1.34[1)]	143.68±11.45[1)]
Vitamin E group	169.80±9.84[2)]	4.87±0.51[2)]	22.37±1.48[2)]	182.43±10.30[2)]
Low dose group	151.36±11.27[2)]	5.74±0.47[2)]	19.21±1.26[2)]	150.72±8.62[2)]
Medium dose group	160.22±8.62[2)]	5.23±0.53[2)]	21.59±1.07[2)]	169.58±9.58[2)]
High dose group	168.93±7.20[2)]	4.98±0.49[2)]	22.09±1.17[2)]	175.87±8.63[2)]

Compared with the normal group: 1) p<0.01; compared with the model aging group: 2) p<0.01

The effect of LBP on SOD activity and MDA content in aging model mouse

Compared with the normal group, mouse skin SOD activity in the model aging group decreases significantly, and that in the drug use groups and the vitamin E group can be increased significantly. Compared with the normal group, MDA content in mouse skin in the model aging group increases, and that in the drug use groups and the vitamin E group can be reduced. Compared with the normal group, Hyp content in mouse skin in the model aging group decreases significantly, and that in the drug use groups and the vitamin E group can be increased, as shown in Table 5.

Table 5: The effect of LBP on SOD, MDA and Hyp in mouse skin

Group	SOD U/mg	MDA nmol/mg	Hyp mg/g
Normal group	253.57±7.50	3.62±0.28	6.75±1.78
Aging group	138.93±6.89[1)]	7.33±0.54[1)]	3.17±0.29[1)]
Vitamin E group	192.74±7.42[2)]	4.13±0.26[2)]	6.08±0.32[2)]
Low dose group	145.62±6.27[2)]	6.03±0.37[2)]	4.57±0.47[2)]
Medium dose group	170.85±5.98[2)]	5.27±0.42[2)]	5.22±0.53[2)]
High dose group	190.23±6.03[2)]	4.58±0.35[2)]	5.92±0.44[2)]

Compared with the normal group: 1) p<0.01; compared with the model aging group: 2) p<0.01

Discussions

The ultrasonic extraction method uses ultrasound to increase the frequency and speed of molecular movement, to increase solvent penetrating power, to increase dissolution rate, to reduce extraction time and to increase the yield. Therefore, this experiment chooses ultrasonic extraction, which is easy and fast, and has a high yield capacity.

After body aging, the contents of SOD, CAT and GSH-PX decrease. Free radical scavenger decreases, and free radical increases and accumulates, causing unsaturated fatty acid oxidation generating a large number of lipid peroxides. SOD is a free radical-clearing Chinese medicine material, and it can clear superoxide anion radicals and protect cells from damage. GSH-px can clear harmful peroxides in cells and cut off lipid peroxidation chain reaction. Hyp is a main component of collagen and collagen fibre and also a relatively stable amino acid. It can stabilise collagen structure and thus repair damaged skin tissue. Lipid peroxide is a product of the adverse effect of free radicals. It can also be used as a quantitative indicator for body and organ aging, and MDA is one of the end products of lipid peroxides (Li, 2006).

The experimental results show that for the mice in the model aging group, cell metabolism is in disorder. Skin SOD activity decreases, MDA content increases, and Hyp content decreases, indicating that skin collagen decreases, fibroblasts arrange loosely, skin elasticity decreases, and skin is at aging state. LBP can increase the contents of SOD, GSH-px, Hyp and CAT. It can equally decrease the content of MDA in the model aging group, indicating that it can increase free radical scavenger activity, inhibit the growth of free radicals, reduce lipid peroxidation and protect tissues from the attack of oxidants and free radicals, thereby exerting its anti-aging effect (Liang, 2009). However, the detailed mechanism is yet to be studied.

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