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Full Length Research Paper

Orthogonal test design for optimization of the extraction of polysaccharides from Lycium barbarum and evaluation of its anti-athletic fatigue activity

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Yield of polysaccharides from Lycium barbarum obtained by hot water and followed by precipitation with ethanol extraction through an orthogonal experiment were investigated to get the best extraction conditions. The results showed that the highest yield was obtained when extraction temperature, extraction time and ratio of solvent to raw material were 93°C, 4.3 h and 43, respectively. The anti-athletic fatigue activities of polysaccharides from Lycium barbarum were evaluated by the weight-loaded swimming model in mice. The results showed that polysaccharides from Lycium barbarum have noticeable anti-athletic fatigue effect on mice. It prolonged the weight-loaded swimming time, prevent the increase of blood lactic acid of mice after swimming and increase the content of hepatic glycogen of mice after swimming. The strongest effect on most biomarkers was seen with 60 mg/kg dose.

Key words: Polysaccharides from lycium barbarum, orthogonal experiment, anti-athletic fatigue, extraction.

INTRODUCTION

Fruit from Lycium barbarum in the family Solanaceae is well-known in traditional Chinese herbal medicine and functional foods which has functions of nourishing the kidney and producing essence, nourishing the liver and brightening eyes, reducing blood glucose and serum lipids, anti-aging, immuno-modulating, anticancer and male fertility-facilitating (Peng et al., 2001; Wang et al., 2001; Wang et al., 2002; Gan and Zhang, 2003). Some constituents of Lycium barbarum fruits have been chemically investigated, especially polysaccharides from Lycium barbarum (LBP) components which are the most important functional factor (Gan and Zhang, 2003; Gan et al., 2004; Qi et al., 2001; Zhang et al., 2005). LBP could be extracted with hot water followed by precipitation with ethanol to obtain high quantity of polysaccharides (Zhang et al., 2004). They have been recently studied for their physiological and pharmaceutical activities. However, there was little research on the effect of LBP on athletic fatigue. In the present experiment, we optimized extraction parameters of LBP by employing an orthogonal test design and evaluated its anti-athletic fatigue activity.

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MATERIALS AND METHODS

Plant materials

Dried Lycium barbarum fruits were purchased from a local drug market and the material was identified by Mr. Zhang Yao, a botanist of Beijing Sport University. A voucher specimen has been deposited in herbarium of Beijing Sport University.

Extraction of LBP

Dried Lycium barbarum fruits were ground in a high speed disintegrator to obtain a fine powder, then were extracted in a Soxhlet apparatus with a mixture of chloroform-methanol (2:1, 75°C) and pretreated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained, as described previously (Zhao and Liu, 2008). The pretreated dry powder (20 g) was extracted with deionized water (solvent-raw material (ml/g) ranging from 20:1 - 60:1) at pH 6.5 - 7.5

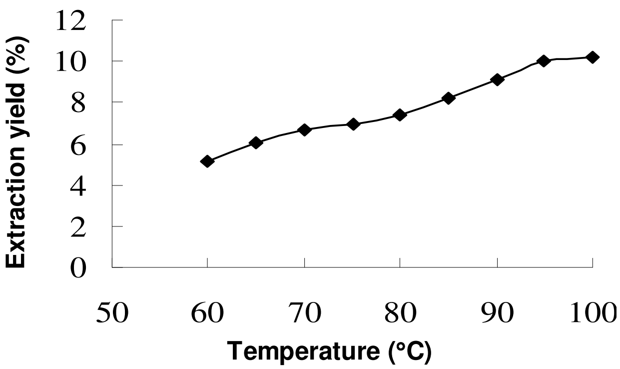

Figure 1. Effect of extraction temperature on extraction yield of LBP.

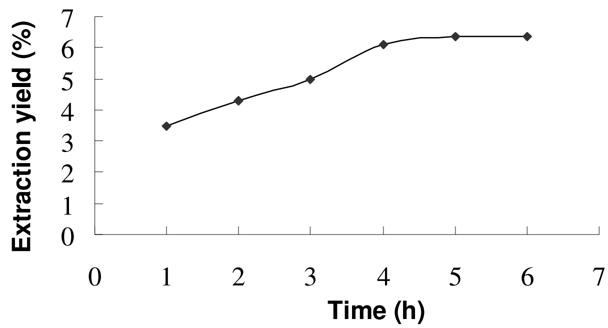

Figure 2. Effect of extraction time on extraction yield of LBP.

(adjusting the suspension pH by 0.1 mol/l NaOH or HCl), while the temperature of the water bath ranged from 60 to 100°C and was kept steady (within ± 1.0°C). The water-Lycium barbarum slurry in a 2.0 L stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 1 - 6 h) during the entire extraction process. The mixture was centrifuged (2000 g, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (pore diameter: 38 um). The extracts were then defatted by the method of Sevag (Sevag et al., 1938), precipitated by the addition of ethanol to a final concentration of 80% (v/v), and the precipitates were collected by centrifugation (2000 g, 20 min), then solubilized in deionized water and lyophilized to get LBP. The contents of polysaccharides were determined by phenol-sulfuric acid method and by reference to glucose, and wavelength in spectrophotometer was set at 490 nm (Wang et al., 2008).

Experimental animals

Kunming male mice with a body weight range of 18 - 22 g were obtained from Center of Experiment Animal of Beijing Sport University. The mice were housed in stainless steel cages at a controlled temperature (22 ± 2°C) and 60 - 65% relative humidity with a normal 12 h light and dark cycle. The study was carried out according to the "Principles of Laboratory Animal Care" (World Health Organization (WHO) Chronicle, 1985). A standard pellet diet and water were given ad libitum. The mice were randomized into three groups equally based on body weight after one week adoption: control group (CG), LBP low dose group (LLG, 20 mg/kg), and LBP high dose group (LHG, 60 mg/kg). LBP were dissolved in distilled water and were fed by gavage to mice once a day for 14 days. The control group was given distilled water and the treated groups were given different doses of LBP.

Anti-athletic fatigue activity

The anti-athletic fatigue activity of LBP was evaluated by the weight-loaded swimming model in mice. The model was a reliable measure of anti-athletic fatigue treatment as established in both laboratory animals and humans (Jung et al., 2007; Jia and Wu, 2008; Selsby et al., 2003). Body weight was recorded before experiment (initial) and after 14 days (final), the average weight-loaded swimming time and corresponding biochemical parameters including serum blood urea nitrogen (BUN), hepatic glycogen and blood lactic acid were observed (Technical Standards for Testing and Assessment of Health Food, Ministry of Health, PR China, 2003). The size of swimming pool was designed as 50 × 50 × 40 cm, filled with fresh water at 30 ± 1°C. A lead block (5% of body weight) was loaded on the tail root of the mouse. The end point of the swimming endurance was taken as when the mouse remained at the bottom for more than 10 s (Zhang et al., 2007).

Biostatistical analysis

All data were analyzed using SPSS software for Windows, which applied the mixed 2 way ANOVA to determine variances. This statistical analysis used $\alpha = 0.05$.

RESULTS AND DISCUSSION

Single factor test for better extraction parameters of LBP

Different extraction conditions, such as extraction temperature, extraction time and ratio of solvent to raw material, have different effects on extraction yield of polysaccharides. As far as the extraction temperature is concerned, the higher the temperature, the higher the extraction yields of LBP. As shown in Figure 1, extraction yield increased from 5.17 - 10.08% with the increasing temperature.

However, on the other hand, a relatively high extraction temperature (at 100°C) was detrimental to the extraction yield. Only a bit of the extraction yield was increased as the temperature was higher than 95°C because of destruction of enzyme activity at high temperature in this reaction system (Gan et al., 2007). Therefore, the suitable temperature for higher yield of the LBP was considered to be 95°C.

Extraction time is another factor that would influence the extraction efficiency (Zhang et al., 2004). With the increase of the extraction time from 1 to 4h in the extraction system, the extraction yield quickly increased from 3.47 - 6.11% (Figure 2). When the extraction time continued to lengthen, the extraction yield increased little. Because longer extraction time could delay and lengthen production cycle, 4h of extraction time was adopted in the present work.

Ratio of solvent to raw material was another factor

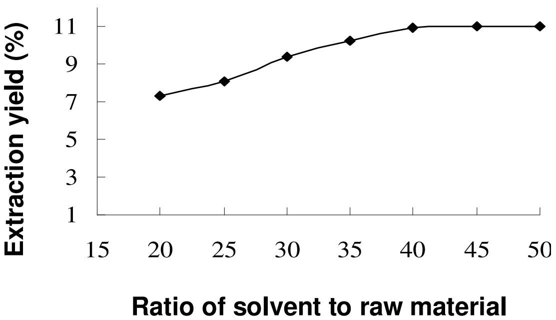

Figure 3. Effect of ratio of solvent to raw material on extraction yield of LBP.

Table 1. Factors and levels of orthogonal test.

Variable	Level		
	1	2	3
A (extraction temperature) (℃)	93	95	97
B (extraction time) (h)	3.7	4.0	4.3
C (ratio of solvent to raw material)	37	40	43

affecting extraction yield of polysaccharides (Zhao and Liu, 2008). The ratio of solvent to raw material was set at 20, 25, 30, 35, 40, 45 and 50 respectively. With the increase of the ratio of solvent to raw material from 20 - 40 in the extraction system, the extraction yield of LBP quickly increased from 7.27 - 10.94 % (Figure 3). It can be seen from Figure 3 that extraction yield of LBPs in the extraction system gives higher value when the ratio of solvent to raw material was 40. In addition, the extraction yield of LBP increased little when the ratio of solvent to raw material surpassed 50. Therefore, the suitable ratio of solvent to raw material for higher total yield of the LBP was considered to be 40.

The orthogonal test for optimal extraction parameters of LBP

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions is a critical step in the development of a solvent extraction method (Liang, 2008). The orthogonal test $L_9(3)^3$ table was designed to detect the most suitable extraction conditions of three factors (extraction temperature, extraction time and ratio of solvent to raw material) for extraction yield (Table 1). According to the value of range R in Table 2, extraction time (factor B) exerted the most significant effect on extraction yield, and the order of importance that influenced extraction yield was found to be extraction time (B) > ratio of solvent to raw material (C) > extraction temperature (A). The optimal combination parameters of the processing technology were $A_1B_3C_3$, namely, extraction temperature (93℃), extraction time (4.3 h) and ratio of solvent to raw material (43).

Anti-athletic fatigue activity of LBP

Effect of LBP on body weight and weight-loaded swimming time of mice are shown in Table 3. There was no significant difference in the body weights of mice in the treated groups (LL group and LH group) compared with C group during initial and terminal stages in the experiment ($p > 0.05$). So the LBP had no significant effect on body weight. The average weight-loaded swimming time of mice of the treated groups was remarkably longer than that of C group ($p < 0.05$), which showed that LBP had significant effect on the endurance of the mice in the experimental and the dosage of 60 mg/kg was more effective.

Effect of LBP on serum blood urea nitrogen (BUN), hepatic glycogen (HG) and blood lactic acid (BLA) of mice are shown in Table 4. The level of serum BUN of the treated groups were lower than that of C group, but there was not significant difference ($P>0.05$), while the level of BLA of the treated were significantly lower than that of C group ($P<0.05$). These results hinted that LBP can effectively retard and lower the blood lactate produced after swimming, postpone the appearance of athletic fatigue and accelerate the recovering from athletic fatigue and the dosage of 60 mg/kg was more effective. The level of hepatic glycogen of the treated groups were higher than that of C group ($p < 0.05$), which indicated that LBP can significantly increase the content of hepatic glycogen of mice after swimming and the dosage of 60 mg/kg was more effective.

It was known that endurance capacity of body was markedly decreased if the energy was exhausted (Kamakura et al., 2007). As glycogen was the important resource of energy during exercise, the increasing of glycogen stored in liver is advantage to enhance the endurance of the exercise (Favier and Koubi, 1988). In our study, the prolongation of the weight-loaded swimming times exhibited by the mice fed with LBP must be related to the improvement in the physiological function or the activation of energy metabolism.

Conclusions

It is considered from these results that LBP has anti-athletic fatigue activity since it prolonged the weight-loaded swimming time, prevent the increase of blood lactic acid of mice and increase the content of hepatic glycogen of mice after swimming. The strongest effect on most biomarkers was seen with 60 mg/kg dose. An evaluation of the pharmacological applications of LBP will provide a more detailed understanding of the factors that enable the LBP to exert its anti- athletic fatigue activity, so more research on the characteristics of LBP will be carried out.

Table 2. $L_9(3)^3$ orthogonal test result.

No.	A (Extraction temperature) (°C)	B (Extraction time) (h)	C (Ratio of solvent to raw material)	Extraction yield (%)
1	1	1	1	9.86
2	1	2	2	11.23
3	1	3	3	11.37
4	2	1	2	10.24
5	2	2	3	11.25
6	2	3	1	10.81
7	3	1	3	10.48
8	3	2	1	10.29
9	3	3	2	11.27
K_1	10.820	10.193	10.320	
K_2	10.767	10.923	10.913	
K_3	10.680	11.150	11.033	
R	0.140	0.957	0.713	

Table 3. Effect of LBP on body weight and weight-loaded swimming time of mice.

Group	Number of animals	Body weight (g)		weight-loaded swimming time(min)
		Initial	Final	
CG	8	24.7 ± 1.4	35.3 ± 3.1	6.87 ± 0.49
LLG	8	24.9 ± 1.2	33.6 ± 2.4	9.64 ± 1.03
LHG	8	24.6 ± 1.3	34.7 ± 2.8	13.5 ± 1.56

Mean ± SD. $p < 0.05$ vs C group.

Table 4. Effect of LBP on serum blood urea nitrogen (BUN), hepatic glycogen and blood lactic acid of mice.

Group	Number of animals	BUN (mmol/L)	HG (mg/g)	BLA (mmol/l)
CG	8	9.87 ± 1.24	7.91 ± 1.12	9.51±1.05
LLG	8	9.11 ± 0.86	12.66 ± 2.84	8.12±0.97
LHG	8	8.92 ± 1.03	15.37 ± 2.47	7.58±0.82

Mean ± SD. $p < 0.05$ vs C group.

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