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Full Length Research Paper

Effects of *Lycium barbarum* polysaccharides (LBP) on immune function of mice

Yi Nan[1], Rong Wang[1], Ling Yuan[2,3] and Yang Niu[1]*

[1]Traditional Chinese Medicine College of Ningxia Medical University,Yinchuan City, Ningxia Province.750004,PR China.
[2]Pharmacy College of Ningxia Medical University, Yinchuan, Ningxia Province.750004, PR China.
[3]Minority Traditional Medical Center of Minzu University of China, Beijing.100081, PR China.

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This experiment was conducted to investigate the effects of *Lycium barbarum* polysaccharides (LBP) on immune system function. The effects of different doses of LBP on the relative weight of immune organs, delayed type hypersensitivity, spleen lymphocyte proliferation reaction and NK cell activity in mice were investigated. LBP increased the relative weight of immune organs and improved cellular immune function, humoral immune function and non-specific immune function in mice. It also significantly improved immune function in mice.

Key words: *Lycium barbarum* polysaccharides (LBP), immune, cell proliferation, NK cell.

INTRODUCTION

Barbary wolfberry fruit is the dry and ripe fruit of *Lycium barbarum* L. in Solanaceae plant (ChP, 2010). Barbary wolfberry fruit is a commonly used traditional Chinese medicine and originally recorded in the ancient Chinese medical classic “Shen Nong’s Herbal Classic” (Dictionary of TCM, 1977). Barbary wolfberry fruit has the effects of nourishing liver and kidney, replenishing vital essence to improve eyesight, moisturizing lungs and relieving cough, slowing down aging, etc. The chemical composition of barbary wolfberry fruit includes carbohydrates (Ni and He, 1993), amino acids (Chen, 2001), trace elements (Wang, 1991), vitamins, superoxide dismutase (SOD) (lv Bingyi and fan, 1991), alkaloids (Wang, 1991), fat and fatty acids, inorganic salt (lv Bingyi and fan, 1991), etc. Barbary wolfberry has high sugar content, with the total sugar content of 46.5% and its main active ingredient is *Lycium barbarum* polysaccharides (LBP) (Zhou and Li, 2009). It has been reported in literatures that LBP is one of glycoconjugates, a conjugate formed by polysaccharides and peptides or proteins and the main active component in barbary wolfberry. It is also known to contain 17 different types of amino acids.

In recent years, with the continuous deepening of the study on the chemical ingredients of barbary wolfberry, many new progresses are made in pharmacological study and clinical application of barbary wolfberry at home and abroad. Modern pharmacological experiments show that: barbary wolfberry has the effects of improving body immune function, inhibiting tumor, lowering blood sugar, lowering blood fat, resisting fatigue, etc. This paper is to investigate the immune activity of LBP and its mechanism of action through studying the regulation effects of LBP on immune system in mice.

MATERIALS AND METHODS

Experimental subjects and reagents

Kunming mice, body weight (20 ± 2) g, half males and half females, were bought from the Institute of Laboratory Animal Sciences of CAMS; Medimachine from Denmark DAKO; MTT and ConA from Sigma; Cyclophosphomide (CY) from Jiangsu HengRui Medicine Co., Ltd.

LBP preparation (Huang et al., 1998)

1000 g barbary wolfberry fruit (*Lycium barbarum* L.) was crushed

*Corresponding author. E-mail: nanyiailing10 @sohu.com. Tel: +86 0951-4091605.

and immersed in water twice (24 and 6 h), the filtrate was combined, concentrated and centrifuged, and the supernatant was precipitated with absolute ethanol. After precipitation and dissolving, 1/5 volume of savage reagent ($CHCl_3$-n-BuOH 4:1) was used to remove free protein (7 times). The drug solution was concentrated to the relative density of 1.3. After freezing and drying, 7 g LBP was obtained for future use.

Grouping

The control group and the test groups

It was randomly divided into four groups, that is, the solvent control group (normal saline) and the LBP test groups (0.5 g, 1 g and 2 g/kg) for intragastric administration once a day for continuous 30 d, body was weighed weekly to adjust the dose, and in the test period, animals ate and drank freely.

The CY group

Each mouse accepted intraperitoneal injection of 200 mg/kg CY on D1, and others were same as the control group.

Mouse spleen lymphocyte suspension preparation (Fatima et al., 2000)

The mice in all the groups were killed by cervical dislocation, spleens were taken sterilely, cut into small pieces, processed into single cell suspension by Medimachine and centrifuged at 1 000 r/min for 5 min, and the supernatant was discarded. After washing with phosphate buffered saline (PBS) twice, the cells were prepared into 5×10^6/ml with RPMI-1640 culture solution containing 10% calf serum, and it was blown evenly for future use.

Effect on mass of immune organs of mice

In the next day after the last drug administration, mice in the LBP groups (low, medium and high dose groups) and the control group were killed by orbit bloodletting and dissected, spleen and thymus were weighed, and the organ index was calculated.

Delayed type hypersensitivity (DTH) test

Sheep red blood cells (SRBC) immunized mice (volume fraction of 2%) were taken 30 d after intragastric administration, the left rear paw thickness was measured after 4 d, then 15 μl SRBC with volume feaction of 20% was subcutaneously injected at the measuring site, the left rear paw thickness was measured 24 h after injection, and the difference of paw thickness before and after attack was calculate, that is, the degree of DTH.

Spleen lymphocyte proliferation test

30 d after intragastric administration, the mice were killed by cervical dislocation, and spleens were taken sterilely, grounded, filtered, washed and prepared into spleen lymphocyte suspension (5×10^6/ml). It was added to 96-pore plate, 100 μL in each pore. ConA (final mass concentration 5 μg/ml) was added in, and blank control was set. It was placed in 37℃ and 5% CO_2 constant temperature incubator to culture for 48 h and 10 μL 5 mg/ml MTT solution was added into each pore to continue culturing for 4 h. After culture, the supernatant was discarded, 150 μL dimethyl sulfoxide (DMSO) was added into it, after standing for 20 min, rate value was measured at 570 nm and the proliferation calculated.

Effect on mouse NK cell activity

30 d after intragastric administration, the mice were killed by cervical dislocation, and spleens were taken sterilely, grounded, filtered, washed and prepared into spleen lymphocyte suspension (22×10^7/ml), 100 μL RPMI-1640 complete medium was added into, that is, effector cells; with YAC-1 cells with the cell concentration of 5×10^6/ml after subculturing as the target cells, effector cells and target cells were cultured in the 37℃ and 5% CO_2 incubator for 4 h, 10 μL MTT solution (5 mg/ml) was added into to continue culturing for 4 h, it was centrifuged for 10 min (3 000 r/min), the supernatant was discarded, 100 μL DMSO was added into each pore, and A value was measured at 570 nm after shaking thoroughly.

RESULTS

Result of the effect on the mass of immune organs in mice

The test proves that the mass of spleen and thymus of mice can be increased in the LBP test groups, and with the increase of dose, the increase of the mass of immune organs in mice is not significant, as shown in Table 1.

Result of delayed type hypersensitivity test

After certain allergen contacts mice, it can stimulate T-lymphocytes to be transformed and proliferated to sensitized lymphocytes, and antigen attack causes local DTH reactions, leading to mouse ear or foot skin swelling. After SRBC attack, the mouse paw thickness increase in the LBP groups is significantly higher than that in the control group, indicating that LBP has the effect of promoting the ability of delayed type hypersensitivity in mice (Table 2).

Effect of LBP on CY immune suppression of mouse lymphocyte proliferation

It can be seen from Table 3 that; compared with the control group, it can significantly improve the ability of spleen lymphocyte proliferation induced by ConA in all the LBP groups, and significant dose-reaction relationship exist among the three dose groups, and linear test is significant, indicating that LBP has the effect of promoting ConA-induced lymphocyte proliferation.

Effect on mouse NK cell activity

The results shows that the LBP dose groups present dose dependence relationship in increasing CY immunosuppressed mouse NK cell activity (Table 4).

Table 1. Effect of LBP on immune organ index in mice.

Group	Dose/(mg·kg^{-1})	Spleen index/(mg·10 g^{-1})	Thymus index/(mg·10 g^{-1})
The control group	0	4.32 ± 0.82	2.93 ± 0.37
The low dose group	50	5.24 ± 0.79**	4.09 ± 0.48
The medium dose group	100	5.78 ± 0.95**	4.73 ± 0.64
The high dose group	200	6.23 ± 0.46**	4.96 ± 0.54

Compared with the control group, **P<0.01.

Table 2. Test result of effect of LBP on mouse foot swelling caused by SRBC.

Group	Dose/(mg·kg^{-1})	DTH paw thickness increase (mm)
The control group	0	0.24 ± 0.07
The low dose group	50	0.42 ± 0.22*
The medium dose group	100	0.47 ± 0.11**
The high dose group	200	0.49 ± 0.16**

Compared with the control group, *P<0.01, **P<0.001.

Table 3. Effect of LBP on CY immune suppression of mouse lymphocyte proliferation.

Group	Dose/(mg·kg^{-1})	Mouse spleen lymphocyte transformation stimulated by ConA (A added value)
The control group	0	0.51 ± 0.13
The model group		0.33 ± 0.15
The low dose group	50	0.49 ± 0.17*
The medium dose group	100	0.58 ± 0.12**
The high dose group	200	0.59 ± 0.11**

Compared with the model group, *P<0.01, **P<0.001.

Table 4. Effect of BLP on CY immunosuppressed mouse NK cell activity.

Group	NK cell activity / (%)
The control group	82.34 ± 4.32
The model group	52.36 ± 4.46
The low dose group	62.45 ± 5.12
The medium dose group	78.64 ± 4.86
The high dose group	90.23 ± 4.87

DISCUSSION

Polysaccharides exist in widely animals, plants and microorganisms. A large number of modern pharmacological and clinical studies have shown that polysaccharide compound is an immunomodulator, with the effects of activating immune receptors and improving body immune, and the studies are mostly about the activity of plant polysaccharides. Due to unique features, in recent years, plant polysaccharides have been widely used clinically to improve body immune ability and enhance body's antioxidant effects, to regulate physiological functions (Li et al., 1996; Li and Zhang, 2004).

LBP, as the main biologic active ingredient in barbary wolfberry, includes neutral sugar, protein and galacturonic acid, in which neutral sugar is composed of six monosaccharides, namely rhamnose, arabinose, xylose, mannose, glucose and galactose (Wang et al., 2008). Modern pharmacological and clinical studies show that it has the effects of immunoregulation, anti-tumor, anti-aging, etc. The chemical composition of barbary wolfberry is complex, and in addition to vitamins, sterols, betaine and various fatty acids, it also contains a variety of pigments, bringing many difficulties in separation and purification of LBP. In this paper, according to the characteristics of LBP, water extraction and alcohol precipitation method is adopted to get the crude product of LBP, and Savage method is adopted to remove protein, to remove impurities in the premise of ensuring the yield of LBP, laying a foundation for the accuracy of the laboratory data.

Immune function is an important guarantee for body to defense and remove various harmful substances, and the core component of the immune system is lymphocytes (Soumya and Melanie, 2010). Lymphocyte is an immunocompetent cell playing a core role in immune response, and it can be classified as T cells, B cells and NK cells according to the functions. T and B lymphocytes, the immune cells playing a core role, can be respectively transformed into lymphoblasts from resting lymphocytes under the stimulation of mitogen ConA fro mitosis and proliferation (Gregori et al., 2003); NK cell (natural killer cell) is the cells with natural killing ability existing in body. Its cytotoxic activity is not stronger than T cells and K cells, but it has fast action, being one of the important non-specific defense functions in body (Francesco et al., 1993). Its characteristics are that it can damage target cells without the stimulation of any antigen and the participation of complement.

According to the existing reports, this study extracts, separates and purifies LBP – the main active substance in barbary wolfberry, and investigates its immuno-modulatory effects. The results show that LBP can increase mouse spleen and thymus mass, suggesting that LBP can enhance non-specific immune function in mice. To further investigate the mechanism of LBP enhancing immune function in mice, this test establishes mouse CY immunosuppression model to prove that within the dose range, LBP in all the dose groups can improve mouse ConA-induced T and B lymphocyte proliferation, and with dose increase, the proliferation capacity gradually increases. NK cell activity enhancing and delayed type hypersensitivity result are similar to the existing reports (Wang et al., 2005).

In conclusion, LBP can significantly enhance body cellular immunity and humoral immunity, with significant immune enhancing effects.

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