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THE EFFECT AND PROTECTION MECHANISM OF LYCIUM BARBARUM POLYSACCHARIDE SUPPLEMENT ON IMMUNITY OF ATHLETES AFTER HIGHLY INTENSE TRAINING

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ABSTRACT

This study aims to discuss the effect and the protection mechanism of lycium barbarum polysaccharide (LBP) supplement after highly intense training on immunity of athletes. We selected 18 experiment athletes from colleges and divided them into the control group (A), pure training group (B) and LBP supplement group (C). Athletes in group B and C went through highly intense training everyday. The athletes in group C took LBP capsules (5 g each day) morning and night everyday, while the athletes in group B took placebos (5 g) morning and night everyday. Athletes in group A did not need any training but took placebos (5 g) morning and night everyday. T-lymphocyte subsets, immune globulin and interleukin-2 (IL-2) activities of athletes were detected before and after the experiment for further analysis.

In the meantime, in order to detect the effect of LBP on immune organs damage caused by highly intense training, we took mice as the research objects and divided them into three groups (group D, E and F) in the same way, thus to detect the spleen and thymus indexes of mice for results analysis. Results showed that, after highly intense training, compared with group A, the specific values of CD4+%, CD4+ and CD8+ of athletes in group B declined remarkably ($p < 0.01$); the specific value of CD8+% also decreased but only slightly ($p > 0.05$). The specific values of CD4+%, CD8+%, CD4+ and CD8+ of athletes in group C increased significantly compared with group A; moreover, compared with group A and B, detected data of immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG) of group C increased remarkably ($p < 0.05$). Compared with group A and B, activities of IL-2 of athletes in group C enhanced substantially. The thymus indexes of mice in group E were lower than that of the other two groups ($p < 0.05$); no significant difference was discovered in spleen and thymus indexes between group F and group D ($p > 0.05$). In conclusion, supplement of LBP can effectively prevent the decrease of immunity of athletes after highly intense training, as well as has significant inhibiting effect on the immune organs damage caused by highly intense training.

1. Introduction

Lycium barbarum is one of the traditional Chinese medicinal materials, and according to the Compendium of Materia Medica, it has various kinds of effects. Moreover, based on researches on lycium barbarum in recent years, we found that the lycium barbarum polysaccharide (LBP) extracted from the dried lycium barbarum after a series of process like

degreasing, etc., had significant effect on human immunity (Castiel and Gueniche, 2010). Some researchers in China, such as Yang Xinbo, et al. (Yang and Huang, 1998) believed that LBP had the hypoglycemic effect. Bian Lun, et al. (Sun et al., 2013) carried out relevant experiments and the results indicated that, the LBP had the recovery effect on acute liver injury induced by carbon tetrachloride as well as an inhibiting effect on growth and proliferation of human cervical cancer and human gastric adenocarcinoma cells; in addition, it could decrease the Methane Dicarboxylic Aldehyde (MDA) and lipofuscin content in the mice body, thus it also had anti-aging effect. Besides, the LBP also has immunomodulatory, anti-hyperlipidemia, hypotensive, anti-fatigue, anti-radiation and hemopoiesis accelerating effects (Edge and Xin, 1996), etc. Therefore, the LBP has become an important kind of additive in health food.

In order to solve the immune suppression and other problems which occur after highly intense training, physical culture workers and researches in China have carried out lots of studies. Some researches indicated that the best method to avoid low immune functions was to supplement nutritional agents after intensive training to avoid exercise-induced immune suppression (Gonçalves et al., 2012). This study was carried out to discuss the effect of LBP supplement on immunity of athletes as well as its protective effect on organ damage of athletes.

2. Experimental objects and methods

2.1. Research objects

We selected 18 athletes who majored in physical education and were (20±2) years old as the research objects. All research objects took part in the experiment voluntarily. After examination, no object had the disease history of metabolism and immune system. Besides, 24 8-week old healthy male mice which weighted (20±2) g were also selected.

2.2. Research methods

Eighteen athletes were divided into the control group (A), pure training group (B) and LBP supplement group (C) randomly, 6 athletes for each group. During training, athletes in group C took the LBP capsules (5 g each day) according to the specification morning and night. Athletes in group A and B took placebos (empty capsules which had the same shape and color as LBP capsules) morning and night. Except the normal diet, all athletes in three groups did not take any other drugs. This experiment lasted one month. After the experiment, 5 ml of venous blood samples were extracted from each athlete and added with heparin anticoagulant and sent to a hospital for detection of lymphocyte subsets, immune globulin concentrations and IL-2 activities. The 24 mice were also divided into three groups (group D, E and F). Mice in group D did not have any exercise and were fed with normal saline (0.9%). Mice in group E were fed with normal saline (0.9%) after exercise. Mice in group F were fed with LBP after exercise, thus to study the effect of LBP on spleen and thymus damage caused by highly intense exercise.

2.2.1. Detection of lymphocyte subsets

According to tagged molecules on the cell surface, a flow Cytometer (produced by American Beckman Coulter, Inc.) as well as the monoclonal antibody (McAb) indirect immunofluorescence were adopted for the detection of specific values of CD4+%/CD8+% and CD4+/CD8+ (Benetatos *et al.*, 2009).

2.2.2. Detection of serum immune globulin

The concentrations of immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG) in serum protein were detected by immunoturbidimetry. A semiautomatic biochemistry analyzer produced by American Beckman Coulter, Inc. was used.

2.2.3. Detection of IL-2 activities

The heparin anticoagulant blood was mixed with an equal amount of phosphate buffer

saline (PBS) and then slowly added with an equal amount of lymphocyte separation medium. After that, the mixed solution was centrifuged in 2000 rpm for 20 min. Then cells in the lymphocyte layer were drawn and washed by PBS twice and added with a moderate amount of Roswell Park Memorial Institute-1640 culture medium (RPMI-1640) (10% fetal calf serum). The number of lymphocytes was counted by microscopic examination and lymphocytes were diluted to $2\times10^6/mL^{-1}$, i.e., lymphocytes suspension (Suo *et al.,* 2015). Then the lymphocytes suspension was added to a 96-well plate, 100 μL for each well. Phytohaemagglutinin (PHA) was then added and the final concentration reached $100\mu g/mL^{-1}$; each group had duplicate wells (Walsh *et al.,* 2003). After three days of culture, the cultural supernatant was collected for detection of IL-2 level using the enzyme-linked immunosorbent assay. The optical density at 570 nm wavelength was measured by the microplate reader and the number of cytokines in the cultural supernatant was calculated.

2.2.4. Detection of spleen and thymus indexes

After the training, the spleen and thymus were separated from the mice and the indexes of spleen and thymus were calculated. Thymus index=thymus weight/mice weight; spleen index=spleen weight/mice weight (unit was mg/g) (Jia *et al.,* 2014).

2.3 Statistical analysis

Software SPSS 15.0 was used to statistically analyze the obtained experimental data. The significant difference level p was 0.05. $p < 0.05$ indicated that the difference had statistical significance.

3. Results and discussions

3.1. The effect of LBP on T- lymphocyte subsets

After the highly intense training, the effect of LBP supplement on T-lymphocyte subsets was shown in Table 1.

Table 1. Effect of LBP supplement on T-lymphocyte subsets

Group	CD4+%	CD8+%	CD4+/CD8+
Control group (A)	50.68±2.25	28.08±2.65	1.80±0.85
Pure training group (B)	46.09±2.04	27.42±2.28	1.68±0.89
LBP supplement group (C)	56.60±3.14	28.54±1.84	1.98±1.71

Note: the CD4+%, CD4+/CD8+ of group C were compared with that of group B, $p < 0.05$

As shown in Table 1, compared with group A, the specific values of CD4+% and CD4+/CD8+ of group B decreased remarkably ($p < 0.01$), indicating that highly intense training could lead to lower immune functions. However, compared with group A and B, specific values of CD4+%, CD8+% and CD4+/CD8+ of group C increased, which increased significantly compared with group B; the difference had no statistical significance ($p > 0.05$). Therefore, LBP had inhibiting effect on the decrease of T-lymphocyte immune functions caused by highly intense training (Dingjuan et al., 2016).

Table 2 Effect of LBP supplement on immune globulin

Group	$IgA/g\cdot L^{-1}$	$IgM/g\cdot L^{-1}$	$IgG/g\cdot L^{-1}$
Control group (A)	1.61±0.43	1.20±0.13	10.93±2.58
Pure training group (B)	1.65±0.34	1.16±0.15	10.72±2.04
LBP supplement group (C)	1.89±0.64	1.24±0.13	11.21±2.42

Note: the $IgA/g\cdot L^{-1}$ and $IgG/g\cdot L^{-1}$ of group C were compared with that of group B, $p < 0.05$.

3.2. The effect of LBP on immune globulin

In Table 2, compared with group A, the IgA concentration of group B increased while the concentrations of IgM and IgG decreased; however, no significant differences were found ($p > 0.05$). Therefore, highly intense training could result in the increase of IgA concentration and the decrease of IgM and IgG concentrations. The IgA concentration of group C was significantly higher than that of group A and B; there was a significant difference ($p < 0.05$). The concentration of IgG of group C was higher than that of the other two groups ($p < 0.05$) and the difference had statistical significance. There was no significant difference between the concentration of IgM of group C and group A and B ($p > 0.05$). Therefore, the results indicated that the LBP could accelerate the synthesis of immune globulin (Luo et al., 2014).

Table 3. Effect of LBP on IL-2 activities

Group	Right after training	2 hours after training	4 hours after training
Pure training group (B)	4.25±0.18	4.19±0.83	3.85±0.14
LBP supplement group (C)	9.10±0.34	14.74±0.67	18.12±0.43

Note: IL-2 activities of the control group A at the same time points were 4.73±0.85, 4.70±0.86 and 4.71±0.83.

3.3 The effect of LBP on IL-2 activities

As shown in Table 3, right after training, 2 hours after training and 4 hours after training, the IL-2 activities of group C increased significantly compared with that of group A, which had statistical significance ($p < 0.01$). The IL-2 activities of group B after training showed a decreasing tendency, but the difference had no statistical significance ($p > 0.05$).

Table 4. Effect of LBP on spleen and thymus of mice

Group	Number	Weight of thymus	Weight of spleen
Mice control group (D)	6	5.122	12.973
Mice pure training group (E)	6	3.846	12.314
Mice LBP supplement group (F)	6	4.756	12.814

Table 5. Effect of LBP on spleen and thymus of mice after highly intense training

Group	Thymus index (average value of thymus/weight)	Spleen index (average value of spleen/weight)
Mice control group (D)	0.259	0.655
Mice pure training group (E)	0.197	0.631
Mice LBP supplement group (F)	0.237	0.638

Note: the weight of mice in each group was the average value: D group was 19.8 g, E group was 19.5 g and F group was 20.1 g.

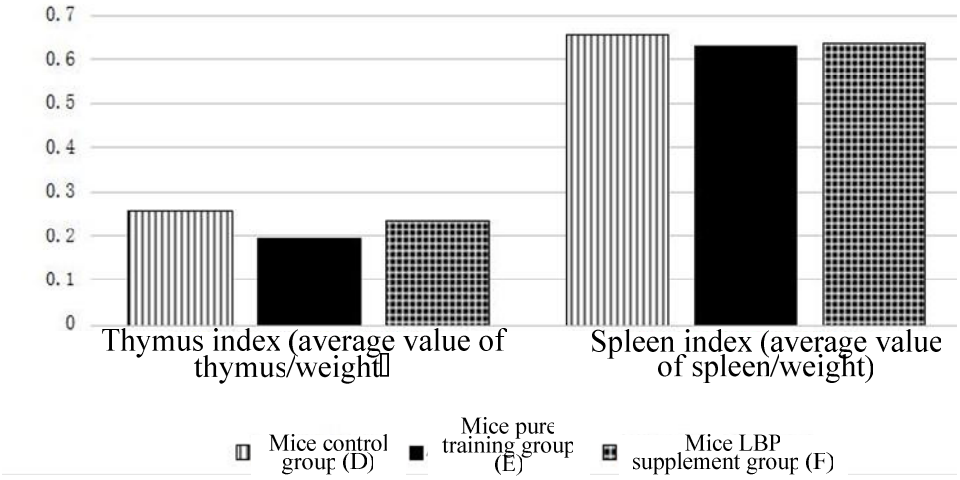

Figure 1. Spleen and thymus indexes of mice after the supplement of LBP

3.4. The effect of LBP on spleen and thymus of mice

The thymus indexes of group E decreased significantly compared with group D ($p < 0.05$), indicating that highly intense training could result in the decrease of thymus weight and thus affect the immune functions. No significant differences were found in spleen indexes and there was no statistical significance. Compared with group D, the indexes of thymus and spleen of group F had no statistical significance ($p > 0.05$). Therefore, the supplement of LBP could recover the immune organ damage caused by highly intense training (So et al., 2011).

As people are paying more and more attentions to the relationship between exercise and immunity, how to enhance the immunity has become an important subject. Highly intense training or competition can result in inhibition of cellular immunity tissues and humoral immunity functions, thus the immunity to pathogenic microorganism can be weakened (Peters, 2004). As an important component of lycium barbarum, LBP contains various kinds of microelements and amino acid (Li et al., 2007), which has significant effect on human immunity.

Results in this study show that, the functions of T-lymphocyte and its subsets are depressed with the increase of training intensity and time. In the experiment, after the supplement of LBP, specific values of CD4+%, CD8+% and CD4+/CD8+ of athletes show increasing tendency, indicating that LBP can improve the proliferation ability of T-lymphocytes. In the experiment of detecting the effect of LBP on immune globulin, the immune globulin is taken as the glycoprotein molecules secreted by activated and proliferated plasmocyte (Jackson and Elsawa, 2015), and its indexes (IgA, IgM, IgG) are important evidences of the effect of LBP on tissues and organs and humoral immunity (Wallukat et al., 2012). Detection of IgA, IgM and IgG in serum shows that, after the supplement of LBP, the values of IgA and IgG increase significantly, indicating that the supplement of LBP can accelerate the synthesis of IgA and IgG and thus improve immunity. Human peripheral blood mononuclear cell IL-2 is a kind of glycoprotein released by helper lymphocytes (Anta et al., 2012), which can accelerate the proliferation of T and B lymphocytes as well as the generation of antibodies. Thus it is an important kind of lymphokine that can improve immunity (Bonini and Bondanza, 2011). LBP can accelerate the synthesis of antibodies through enhancing activities of IL-2 (Corrigall et al., 2004). Moreover, LBP also has recovery and protective effect on damaged thymus caused by intensive training. In experiment of mice, although the

thymus indexes of mice that receive LBP supplement are lower than mice that do not have training, the difference is not significant. Therefore, the supplement of LBP can reduce the damage of thymus caused by highly intense training (Yan et al., 2002).

In conclusion, the supplement of LBP can inhibit the decrease of immune functions caused by highly intense training, as well as effectively relieve and control the body infection caused by massive exercise. In addition, it has a powerful accelerating effect on immunity of athletes and an inhibiting and protective effect on damaged organs caused by highly intense training.

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