

# Therapeutic Effects of *Lycium barbarum* Polysaccharide (LBP) on Irradiation or Chemotherapy-Induced Myelosuppressive Mice

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## ABSTRACT

**Aim:** The aim of this study was to investigate the effects of *Lycium barbarum* polysaccharide (LBP) on irradiation- or chemotherapy-induced myelosuppressive mice and cultured peripheral blood mononuclear cells (PBMCs). **Methods:** In an in vivo experiment, mice were irradiated with a sublethal dose of 550 cGy X-ray or intraperitoneally (i.p.) injected with carboplatin (CB) 125 mg/kg to produce severe myelosuppression. Four to 6 hours after the irradiation or injection, mice were subcutaneously (s.c.) injected with LBP (50, 100, and 200 mg/kg) daily from day 0 to day 6. Blood samples were collected from the tail veins of mice at different time points, and peripheral white blood cells (WBC), red blood cells (RBC), and platelet (PLT) counts were monitored. In an in vitro experiment, human PBMCs were incubated with LBP at different concentrations in combination with phytohemagglutinin (PHA), and the production of granulocyte colony-stimulating factor (G-CSF) was tested. **Results:** Compared to the control, 50 mg/kg LBP (LBP-L) significantly ameliorated the decrease of peripheral WBC of irradiated myelosuppressive mice on day 13, and 100 mg/kg LBP (LBP-M) did the same on days 17 and 21. All dosages of LBP significantly ameliorated the decrease of peripheral RBC of irradiated myelosuppressive mice on days 17 and 25. Two-hundred mg/kg LBP (LBP-H) and LBP-M significantly enhanced peripheral PLT counts of irradiated myelosuppressive mice on days 10, 13, 17, and 21, as did LBP-L on days 13 and 17. All dosages of LBP increased peripheral WBC counts of chemotherapy-induced myelosuppressive mice to some extent, but there was no statistic difference when compared to the control. LBP-H significantly ameliorated the decrease of peripheral RBC of chemotherapy-induced myelosuppressive mice on days 13, 15, 17, and 20, and LBP-M and LBP-L did the same on days 15 and 17. All dosages of LBP significantly enhanced peripheral PLT counts of chemotherapy-induced myelosuppressive mice on days 7 and 10, as did LBP-H on days 13, 15, and 17, and LBP-M on days 13 and 15. Also, LBP could obviously stimulate human PBMCs to produce G-CSF. **Conclusions:** LBP promoted the peripheral blood recovery of irradiation or chemotherapy-induced myelosuppressive mice, and the effects may be the result of the stimulation of PBMCs to produce G-CSF.

**Key words:** *Lycium barbarum* polysaccharide, irradiation, chemotherapy, myelosuppression

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## INTRODUCTION

*Lycium barbarum* polysaccharide (LBP) is the major active principle of a traditional Chinese

herb, *Lycium barbarum* L. The pharmacological effects of LBP have been widely studied, such as its effects on hyperglycemia, hyperlipidemia, and immune modulation.<sup>1-3</sup> LBP is able to regulate the functions of T-cells, B-cells, cytotoxic T-lymphocytes (CTL), natural killer cells and macrophages, improves immune surveillance, and relieves myelotoxicity produced by cancer chemotherapy.<sup>3,4</sup> LBP shows dual-direction regulating effects on interleukin-2 (IL-2) and interleukin-3, and is able to promote IL-2 receptor expression and induce the production of interleukin 6 and tumor necrosis factor.<sup>4</sup> Water extracts of *Lycium barbarum* can promote the immune function recovery of irradiated mice. The indexes of the thymus and spleen of mice, including the proliferation of spleen cells to concanavalin (Con A) or lipopolysaccharide (LPS), mixed lymphocyte reaction (MLR), delayed-type hypersensitive reaction (DTH) and plaque-forming unit counts (PFC), were enhanced by LBP treatment 30 days post-<sup>60</sup>Co radiation.<sup>3</sup> In normal mice, it has been demonstrated that LBP was able to stimulate erythropoiesis and the production of colony-stimulating factors (CSF) to some extent.<sup>5</sup> In our study, we examined the therapeutic effects of LBP on myelosuppressive mice induced by irradiation or carboplatin chemotherapy and cultured human peripheral blood mononuclear cells (hPBMCs). We found that LBP could promote the peripheral blood recovery of irradiation or chemotherapy-induced myelosuppressive mice *in vivo*, as well as stimulate human PBMCs to produce granulocyte colony-stimulating factor (G-CSF) *in vitro*.

## MATERIALS

### Animals

Female Balb/C mice, weighing from 20 to 22 grams and 6 weeks of age, were purchased from the experimental animal center of the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and housed in autoclaved cages in specific pathogen-free rooms. The mice were feeding with sterilized commercial rodent chow and acidified water *ad libitum* in the experimental animal center of the Chinese Academy of Military Medicine (Beijing, China). Animal rooms were maintained at 22°C and 50% relative humidity.

The light cycle was set for 12 hours, beginning from 6:00 AM.

### PBMCs

Human PBMCs were separated from a total of 200 mL of blood donated by a 26-year-old healthy male Chinese volunteer.

### Drug and Reagents

LBP was extracted from herbal *Lycium barbarum* L. produced from the Ningxia province. The LBP preparation was purified with sphadex-G100 columns and was provided by the Department of Chemistry, Pharmagenesis (Beijing, China). The LBP preparation was a water-soluble white powder, and the bacteria and endotoxin was eliminated according to the literature.<sup>6</sup> The average molecular weight of the LBP preparation was 40 kDa, and it was detected by gel filtration chromatography with UV spectrophotometry at 491.5 nm.<sup>7</sup>

Carboplatin (CB), phytohemagglutinin (PHA), RPMI 1640 medium, and fetal calf serum (FCS) were purchased from SIGMA (St. Louis, MO). G-CSF enzyme-linked immunosorbent assay (ELISA) test kits were purchased from Phar Mingen.

### Equipment

A MEK 5108K hematometer and blood sample diluents were purchased from Nihon Kohden Systems (Tokyo, Japan). The X-ray medical radiator was produced by SIEMENS, Germany.

## METHODS

### Myelosuppressive Mice Induced by X-Ray Irradiation and Their Treatment by LBP

According to what had been described,<sup>8,9</sup> mice were divided into 5 groups, including normal group, control group, LBP-L (LBP low dose, 50 mg/kg/body weight) group, LBP-M (LBP middle dose, 100 mg/kg/body weight) group, and LBP-H (LBP high dose, 200 mg/kg/body weight) group, and there were 10 mice in each group. Except for the normal group, all the other mice were put into special cages and irradiated with an X-ray at the sublethal dosage of 550 cGy. Four hours after irradiation, LBP was dissolved in sterilized 5% glucose solution, and was injected subcutaneously (s.c.) into mice daily at the dosages

of 50, 100, or 200 mg/kg/body weight for 7 days. The injection volume of LBP was 0.2 mL per 20 grams of body weight. Mice of the control and normal groups were not treated with LBP.

### Myelosuppressive Mice Induced by CB Chemotherapy and Their Treatment by LBP

Mice were divided into 5 groups, as described above, and myelosuppressive mice were induced according to what had been described.<sup>10</sup> Except for the normal group, all the other mice were intraperitoneally (i.p.) injected with CB at the dosage of 125 mg/kg/body weight. CB was dissolved in normal saline, and the injection volume was the same as for LBP. Four hours after the injection, LBP was injected s.c. to mice daily for 7 days. Mice of the control and normal groups were not treated with LBP.

### Collection and Test of Blood Samples

A 10- $\mu$ L blood sample of each mouse was drawn from the tail vein into an ethylenediaminetetraacetic acid (EDTA)-coated capillary tube on days 7, 10, 13, 17, 21, 25, and 30 in the irradiation experiment, or on days 2, 4, 6, 8, 10, 13, 15, 17, 20, and 24 in the chemotherapy experiment. The samples were mixed with a 10-mL dilution buffer. Peripheral white blood cells (WBC), red blood cells (RBC), and platelets (PLT) were counted by a hematometer.

### PBMCs Separation and G-CSF Assay

PBMCs were separated from healthy total blood, as described.<sup>11-14</sup> PBMCs were cultured

in a 24-well culture plate at the concentration of  $2 \times 10^6$  cells/mL, and were stimulated with 100  $\mu$ g/mL PHA alone or with 100  $\mu$ g/mL of PHA together with various concentrations of LBP for 24 hours. Then the supernatants were collected and the G-CSF levels were detected by ELISA.

### Statistical Analysis

Data were expressed as the mean  $\pm$  standard deviation, and one-way analysis of variance (ANOVA) in SPSS 11.0 was used to analyze the significant difference between the control and LBP treatment groups.

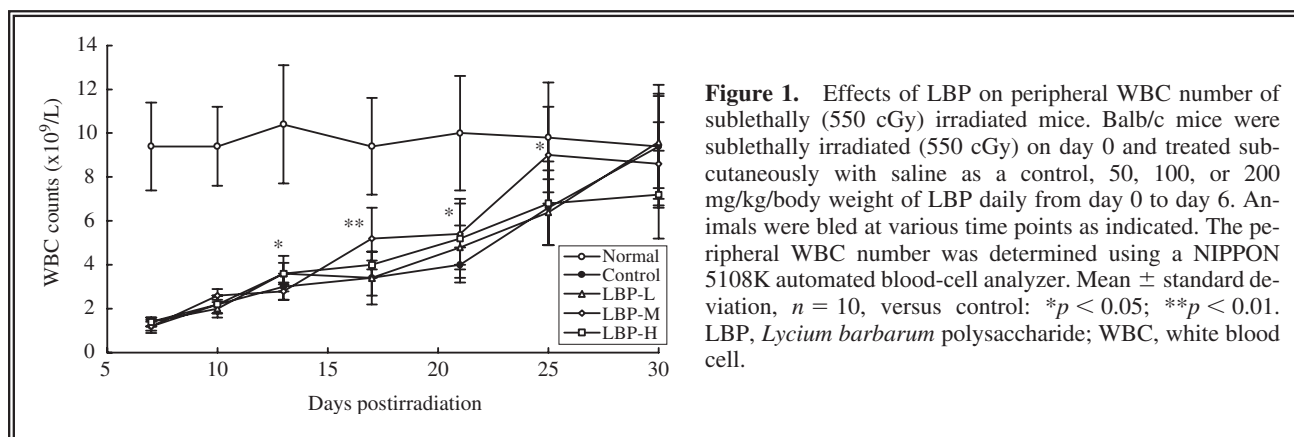
## RESULTS

### Effects of LBP on Irradiation-Induced Myelosuppressive Mice

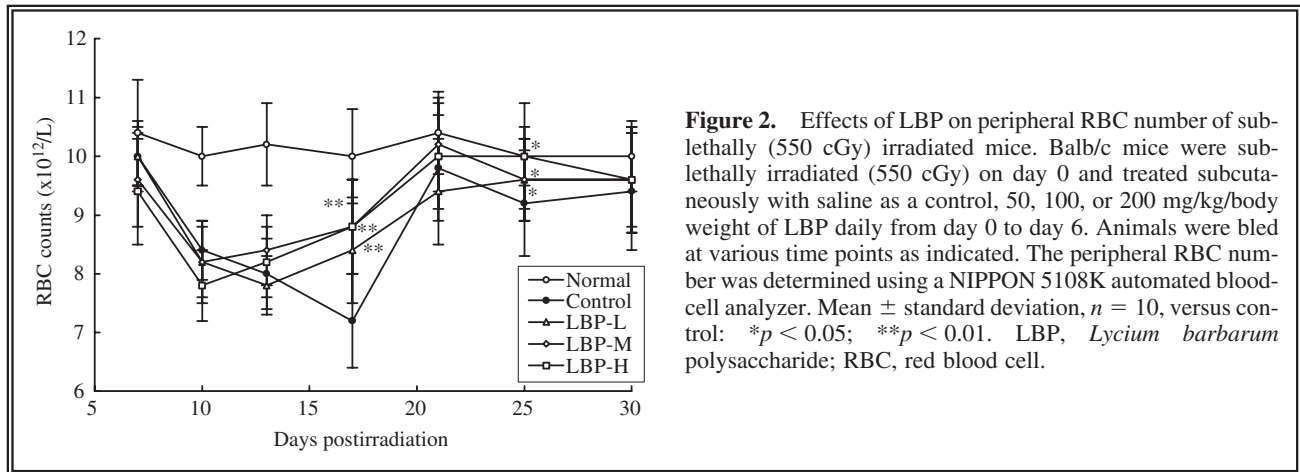
To study the effects of LBP on irradiation-induced myelosuppressive mice, we treated the irradiated mice with LBP at different dosages and tested the peripheral WBC, RBC, and PLT of mice blood.

As shown in Figure 1, the number of peripheral WBC decreased markedly after irradiation and recovered 30 days later. Compared to the control, LBP-H on day 13, LBP-M on days 17, 21, and 25 significantly ameliorated the decrease of peripheral WBC of irradiated myelosuppressive mice.

Similar to what has been reported,<sup>8,9</sup> the number of peripheral RBC decreased markedly after irradiation, reached the lowest level on day 17, and began to recover on day 21. As shown in Fig-



**Figure 1.** Effects of LBP on peripheral WBC number of sublethally (550 cGy) irradiated mice. Balb/c mice were sublethally irradiated (550 cGy) on day 0 and treated subcutaneously with saline as a control, 50, 100, or 200 mg/kg/body weight of LBP daily from day 0 to day 6. Animals were bled at various time points as indicated. The peripheral WBC number was determined using a NIPPON 5108K automated blood-cell analyzer. Mean  $\pm$  standard deviation,  $n = 10$ , versus control: \* $p < 0.05$ ; \*\* $p < 0.01$ . LBP, *Lycium barbarum* polysaccharide; WBC, white blood cell.



ure 2, all dosages of LBP significantly ameliorated the decrease of peripheral RBC of irradiated myelosuppressive mice on days 17 and 25.

As has been reported,<sup>8,9</sup> the number of peripheral PLT of mice decreased markedly after irradiation, reached the lowest level on day 10, and began to recover 25 days later. As shown in Figure 3, LBP-H and LBP-M significantly enhanced peripheral the PLT number of irradiated myelosuppressive mice on days 10, 13, 17, and 21 as did LBP-L on days 13 and 17.

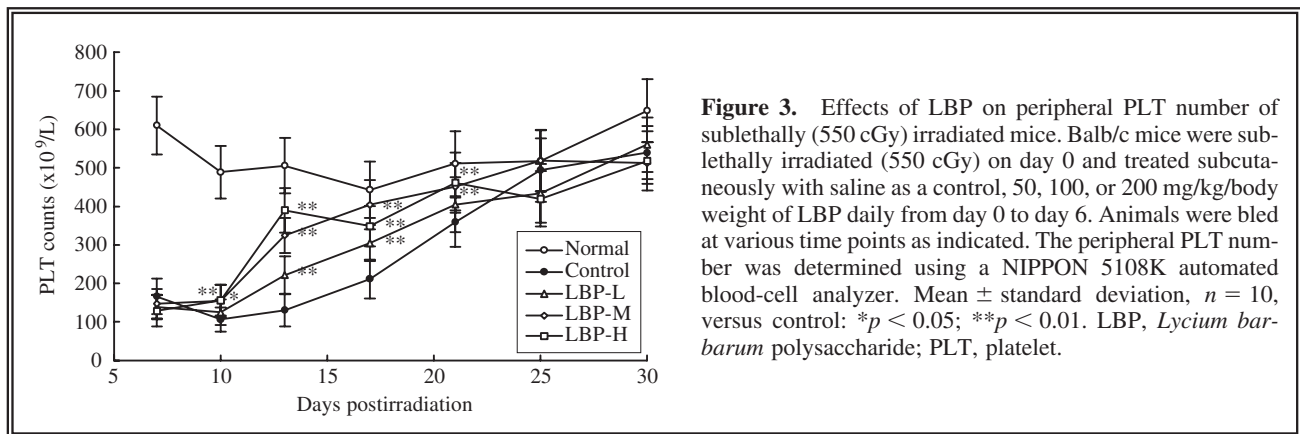
### Effects of LBP on Chemotherapy-Induced Myelosuppressive Mice

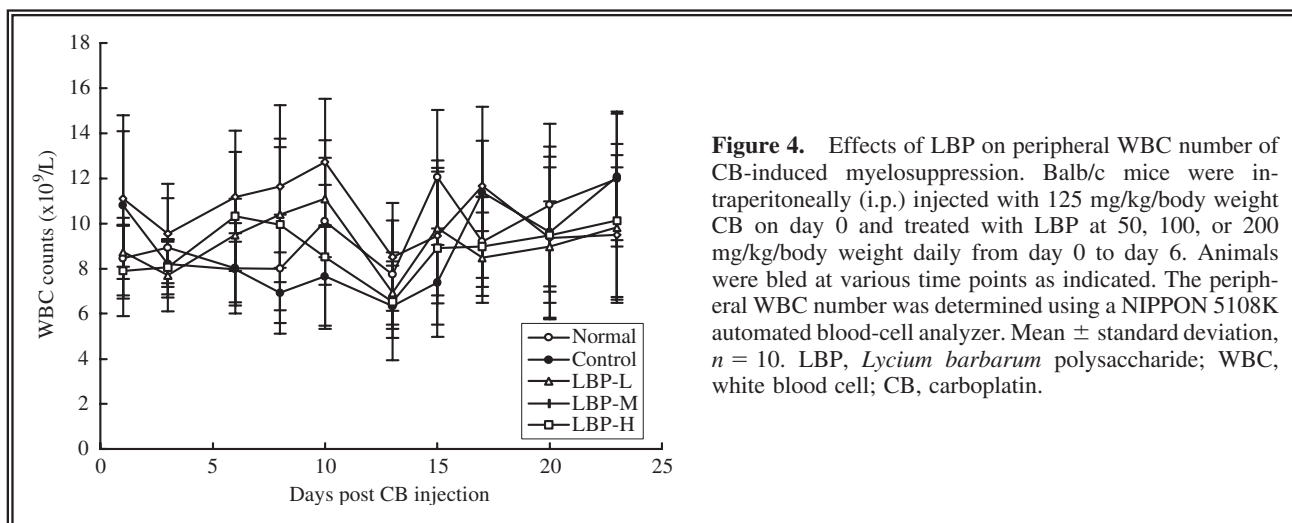
To study the effects of LBP on chemotherapy-induced myelosuppressive mice, we treated the CB-induced mice with LBP at different dosages and tested the peripheral WBC, RBC, and PLT of mice blood.

As shown in Figure 4, the number of peripheral WBC of mice after CB chemotherapy was not markedly decreased, and all dosages of LBP could increase the peripheral WBC number of chemotherapy-induced myelosuppressive mice to some extent, but there was no significant statistical difference.

As reported,<sup>10</sup> the number of peripheral RBC of mice markedly decreased after CB chemotherapy, reached the lowest level on day 10, and began to recover on day 24. As shown in Figure 5, LBP-H significantly ameliorated the decrease of peripheral RBC of chemotherapy-induced myelosuppressive mice on days 13, 15, 17, and 20, as did LBP-M and LBP-L on days 15 and 17.

As previously reported,<sup>10</sup> the number of peripheral RBC of mice markedly decreased after CB chemotherapy, reached the lowest level on day 15, and began to recover on day 17. As shown in Figure 6, LBP-H significantly ameliorated the de-





**Figure 4.** Effects of LBP on peripheral WBC number of CB-induced myelosuppression. Balb/c mice were intraperitoneally (i.p.) injected with 125 mg/kg/body weight CB on day 0 and treated with LBP at 50, 100, or 200 mg/kg/body weight daily from day 0 to day 6. Animals were bled at various time points as indicated. The peripheral WBC number was determined using a NIPPON 5108K automated blood-cell analyzer. Mean  $\pm$  standard deviation,  $n = 10$ . LBP, *Lycium barbarum* polysaccharide; WBC, white blood cell; CB, carboplatin.

crease of peripheral RBC of chemotherapy-induced myelosuppressive mice on days 13, 15, 17, and 20, as did LBP-M and LBP-L on days 15 and 17.

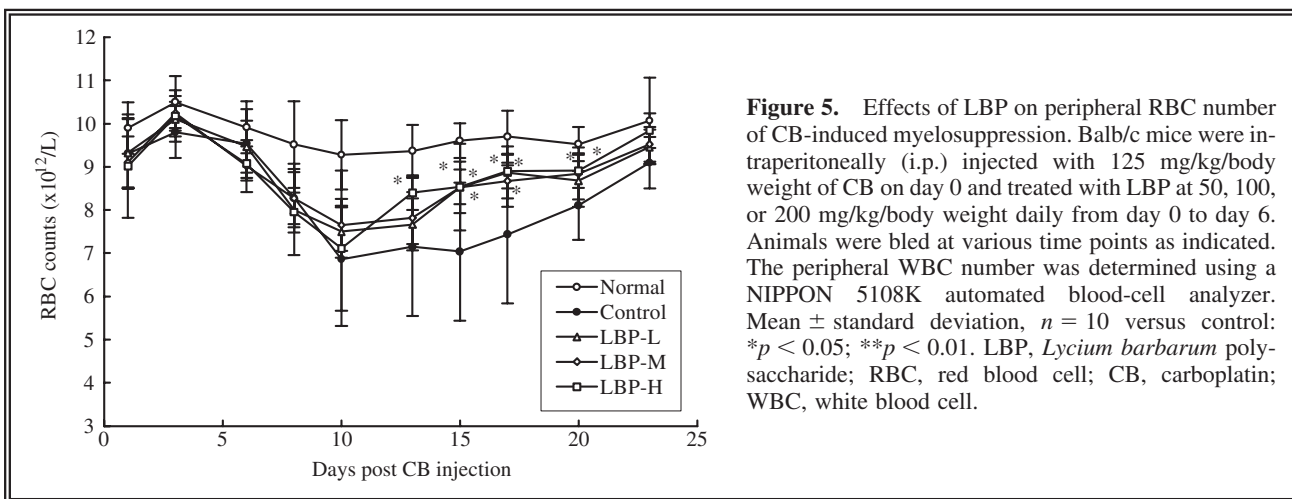
#### Effects of LBP on Human PBMCs

To study the effects of LBP on human PBMCs, we treated human PBMCs with 100  $\mu\text{g}/\text{mL}$  PHA alone or in combination with LBP at 62.5, 125, 250, 500, and 1000  $\mu\text{g}/\text{mL}$  separately, and the production of G-CSF was tested.

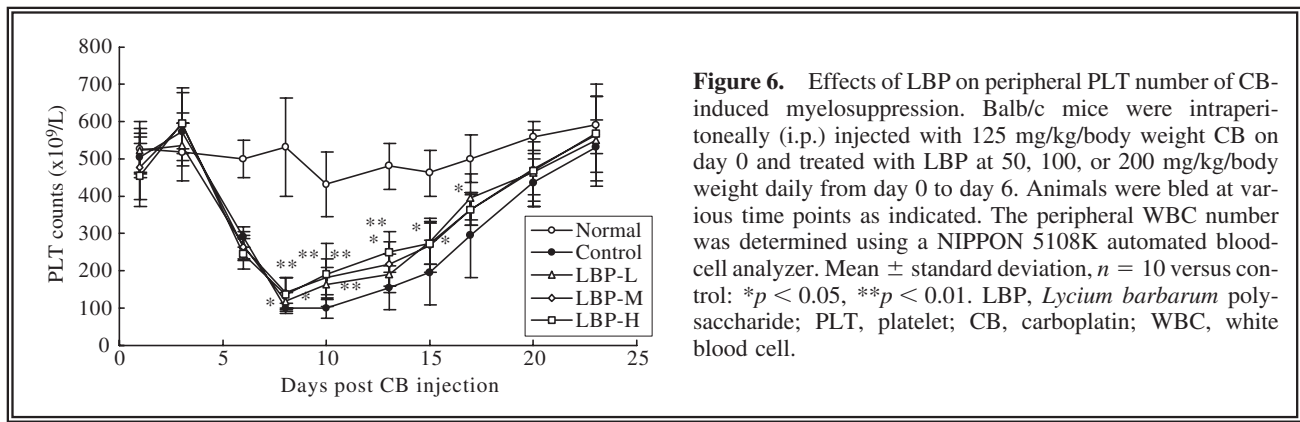
As shown in Figure 7, the G-CSF production was markedly increased after the treatment of LBP in combination with PHA, and all concentrations of LBP could significantly increase the G-CSF production.

#### DISCUSSION

Myelosuppression is an important limiting factor on the outcome and recovery of a tumor patient receiving chemotherapy. In the clinic, G-CSF has been used to improve peripheral neutrophils, erythropoietin (EPO) to promote the production of erythrocytes, and thrombopoietin (TPO) to treat thrombocytopenia. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is 1 of 4 major colony-stimulating factors (CSFs) that regulate hematopoiesis. GM-CSF can stimulate a single bone marrow stem cell to proliferate and differentiate into mature neutrophils, eosinophils, granulocytes, or macrophages.<sup>15</sup> However, the indications of the above regimens are limited be-



**Figure 5.** Effects of LBP on peripheral RBC number of CB-induced myelosuppression. Balb/c mice were intraperitoneally (i.p.) injected with 125 mg/kg/body weight of CB on day 0 and treated with LBP at 50, 100, or 200 mg/kg/body weight daily from day 0 to day 6. Animals were bled at various time points as indicated. The peripheral WBC number was determined using a NIPPON 5108K automated blood-cell analyzer. Mean  $\pm$  standard deviation,  $n = 10$  versus control: \* $p < 0.05$ ; \*\* $p < 0.01$ . LBP, *Lycium barbarum* polysaccharide; RBC, red blood cell; CB, carboplatin; WBC, white blood cell.



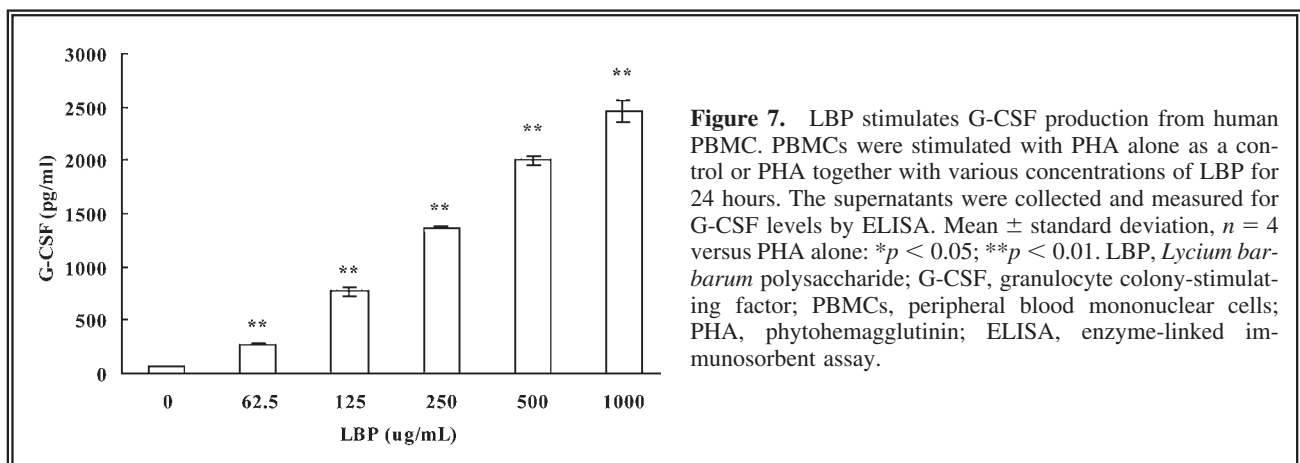
**Figure 6.** Effects of LBP on peripheral PLT number of CB-induced myelosuppression. Balb/c mice were intraperitoneally (i.p.) injected with 125 mg/kg/body weight CB on day 0 and treated with LBP at 50, 100, or 200 mg/kg/body weight daily from day 0 to day 6. Animals were bled at various time points as indicated. The peripheral WBC number was determined using a NIPPON 5108K automated blood-cell analyzer. Mean  $\pm$  standard deviation,  $n = 10$  versus control: \* $p < 0.05$ , \*\* $p < 0.01$ . LBP, *Lycium barbarum* polysaccharide; PLT, platelet; CB, carboplatin; WBC, white blood cell.

cause of their adverse effects and their high costs. Since the early 1970s, herbal polysaccharides has begun to attract the attention of pharmaceutical scientists. Currently, therapeutic polysaccharides derived from traditional Chinese medicines have been widely used. Injectable *Astragalus* polysaccharide (APS) has been shown to enhance the proliferation and maturation of peripheral blood progenitor cells in mitomycin C (MMC)-treated mice,<sup>16</sup> and it has been approved with the functions of immune stimulation and neutrophil enhancement in China.

Chinese herbal *Lycium barbarum L.* is one of the commonly used drugs in traditional Chinese medical practice. It is effective in strengthening the kidney, replenishing vital essence, nourishing the liver to improve the acuity of vision, and moistening of the lungs. Modern research has found that it has a number of effects, such as hyperglycemic, hyperlipid, and immune modula-

tion, and so forth. The pharmacological action of *Lycium barbarum L.* on hematopoiesis has also been reported. For example, LBP can promote erythropoiesis and CSF production in normal mice.<sup>5</sup> Researchers have found that 100% water extracts of *Lycium barbarum L.* could significantly promote the proliferation of bone marrow cells and enhance peripheral WBC counts of <sup>60</sup>Co-irradiated mice.<sup>17</sup> LBP has also been shown to promote the proliferation of monocytes in irradiated mice.<sup>18</sup>

Experimental myelosuppressive animal models are usually induced by chemotherapy or irradiation, and it has been widely used in evaluating the effects of hematopoiesis stimulants. As described in various methods,<sup>8-10</sup> this study used 125 mg/kg carboplatin chemotherapy or 550 cGy X-ray irradiation and successively reproduced the myelosuppressive mice with similar myelosuppressive results, though the ani-



**Figure 7.** LBP stimulates G-CSF production from human PBMC. PBMCs were stimulated with PHA alone as a control or PHA together with various concentrations of LBP for 24 hours. The supernatants were collected and measured for G-CSF levels by ELISA. Mean  $\pm$  standard deviation,  $n = 4$  versus PHA alone: \* $p < 0.05$ ; \*\* $p < 0.01$ . LBP, *Lycium barbarum* polysaccharide; G-CSF, granulocyte colony-stimulating factor; PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin; ELISA, enzyme-linked immunosorbent assay.

mal species were different from the literature.<sup>8-10</sup> Considering that the total volume of blood circulation of the mice was less than 2 mL and the injection volume of LBP was approximately 0.2 mL, which was nearly 10% of the total blood. We selected the s.c. injection for LBP in order to keep the blood pressure from increasing.

## CONCLUSIONS

Our results demonstrate that LBP has therapeutic effects on both irradiation- and chemotherapy-induced myelosuppressive mice, including promoting the recovery of RBC and PLT, and mild effects on WBC. To date, there have been few reports about the effects of plant polysaccharides on G-CSF production. The effects of LBP on stimulating human PBMCs to produce G-CSF may be one of the more important pharmacological factors of promoting hematopoiesis. The extraordinary properties of polysaccharides provide new hope for patients with severe erythropenia and/or thrombocytopenia caused by chemotherapy or radiation. However, there are still many problems that need to be resolved concerning plant polysaccharides, which include that it is usually hard to be solved in solutions, and it sometimes causes adverse reactions to patients, such as hypersensitive reaction or allergy. Many researchers have reported the anaphylactic reactions caused by *Polyporus umbellatus* polysaccharides or lentinan to patients in China.<sup>19-23</sup> In general, effective polysaccharides on hematopoiesis which are safe remain to be developed.

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