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Cordyceps militaris polysaccharides can enhance the immunity and antioxidation activity in immunosuppressed mice

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ABSTRACT

To evaluate the immune activation and reactive oxygen species scavenging activity of *Cordyceps militaris* polysaccharides (CMP) in vivo, 90 male BALB/c mice were randomly divided into six groups. The mice in the three experimental groups were given cyclophosphamide at 80 mg/kg/d via intraperitoneal injection and 17.5, 35, or 70 mg/kg body weight CMP via gavage. The lymphocyte proliferation, phagocytic index, and biochemical parameters were measured. The results show that the administration of CMP was able to overcome the CY-induced immunosuppression, significantly increased the spleen and thymus indices, and enhanced the spleen lymphocyte activity and macrophage function. CMP can also improve the antioxidation activity in immunosuppressed mice, significantly increase the superoxidase dismutase, catalase, and glutathione peroxidase levels and the total antioxidant capacity, and decrease the malondialdehyde levels in vivo.

1. Introduction

In recent years, many natural polysaccharides and polysaccharide-protein complexes were isolated from fungi and used as a source of therapeutic agents (Novak & Vetvicka, 2008). Among them, *Cordyceps militaris*, an entomopathogenic fungus belonging to the class Ascomycetes, has been extensively used as a crude drug and a folk tonic food in East Asia. *C. militaris* is known as the Chinese rare caterpillar fungus and has similar pharmacological activities to the well-known Chinese traditional medicine *Cordyceps sinensis* (Gai, Jin, Wang, Li, & Li, 2004; Zheng & Cai, 2004). The beneficial effects of Cordyceps on renal and hepatic functions and immunomodulation-related antitumour activities are most promising and deserve further attention (Paterson, 2008).

Various bioactive constituents from the Cordyceps species have been reported, such as cordycepin, polysaccharides, antibacterial and antitumour adenosine derivatives, ophicordin, an antifungal agent, and L-tryptophan. Polysaccharides are considered one of the major active components of Cordyceps. Purified polysaccharides from *C. militaris* have numerous biological activities, such as antioxidant (Li, Li, Dong, & Tsim, 2001; Li et al., 2003), immunomodulatory (Cheung et al., 2009; Kim et al., 2008), antitumour (Park, Kim, Lee, Yoo, & Cho, 2009; Rao, Fang, Wu, & Tzeng, 2010), and anti-inflammatory (Rao et al., 2010).

Previous studies on the immunomodulatory and antioxidant effects of *C. militaris* polysaccharides (CMPs) in in vitro systems have been conducted. CMPs can induce the functional activation of macrophages through the upregulation of cytokine expression and nitric oxide (NO) release (Lee et al., 2010), induce T-lymphocyte proliferation and secretion of interleukin (IL)-2, IL-6, and IL-8 (Chen, Zhang, Shen, & Wang, 2010), and stimulate the phagocytosis of macrophages in vitro. These results confirm the important role of CMPs in triggering immune responses. The CMPs fractions P70-1 and CBP-1 were found to exhibit hydroxyl radical-scavenging activity in vitro (Yu et al., 2007, 2009).

In the present study, the fruiting body of *C. militaris* came from Shanghai, which has been scarcely investigated. Successive tests were conducted to evaluate the immune activation and reactive oxygen species (ROS)-scavenging activity of CMP in vivo. The details are reported in the current study.

2. Materials and methods

2.1. Material

Dry cultured *C. militaris* was obtained from Shanghai Dianzhi Bioengineering Corp. (Shanghai, China). The material (No. 06-01-0727) was identified by Associate Researcher X.H. Gao of the Research Group of Dong Chong Xia Cao, Shanghai

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Academy of Agricultural Sciences, Shanghai, China. RPMI 1640 was purchased from Gibco. The T-cell mitogen concanavalin A (ConA) was purchased from Sigma. Dimethyl sulfoxide (DMSO) was acquired from the Yixin Institute of Chemical Engineering (Jiangsu, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco Co. Assay kits for the total antioxidant capacity (TAOC), malondialdehyde (MDA), catalase (CAT), superoxidase dismutase (SOD), and glutathione peroxidase (GSH-Px) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Cyclophosphamide (CY) was purchased from Jiangsu Hengrui Medicine Co., Ltd. (Lianyungang, Jiangsu, China). Bovine serum albumin, Coomassie Brilliant Blue G-250, and cellulose sacks were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The filter membrane was purchased from Millipore Corp. (Billerica, MA, USA). All chemicals used in the experiments were of analytical grade.

2.2. Polysaccharide extraction

Polysaccharides from *C. militaris* were prepared as previously described (Li, Yang, & Tsim, 2006; Yu et al., 2007). The dried powder of cultured *C. militaris* was defatted with ethanol for 10 h and subsequently extracted three times with hot water (100 °C) for 2 h. The resulting suspension was centrifuged (8000 × *g* for 20 min) and filtered through a 0.45 μm membrane (Millipore). The filtered aqueous solution was concentrated to a specific volume under reduced pressure. The dark brown precipitate was collected via centrifugation and washed twice with ethanol. The precipitate was then suspended in water and lyophilized to yield CMP with 41.2% (w/w) polysaccharide content, which was measured using vitriol-anthrone with anhydrous glucose as the standard control.

2.3. Animal and experimental design

Male BALB/c mice (8 weeks old, 18 h to 20 g) were purchased from Shanghai Slac Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China). The animals were provided with water and mouse chow ad libitum and were housed in a rodent facility at (22 ± 1) °C with a 12 h light-dark cycle for acclimatization. All procedures involving animals and their care were approved by the Ethics Committee of the Chinese Academy of Agricultural Sciences. The mice were randomly divided into 6 groups consisting of 15 mice each. Three mice from each group were used for phagocytic index determination in the carbon clearance test, 3 were used for lymphocyte proliferation, and 9 were used for the other experiments. All animals were allowed one week to adapt to their environment before the treatment. Two groups of healthy mice were used as normal control (NS group) and positive control groups and treated once daily with physiological saline solution and 70 mg/kg body weight CMP, respectively, for 18 days. From days 1 to 3, the other four groups of mice were given 80 mg/kg/d CY via intraperitoneal injection. From days 4 to 18, the mice were administered as follows: model group, physiological saline solution; three CMP groups, 17.5, 35, or 70 mg/kg body weight CMP. CY (0.2 ml) was administered via intraperitoneal injection. The others were administered via gavage in 0.2 ml solutions. Twenty-four hours after the last drug administration, the animals were weighed and then sacrificed via decapitation. The heart, liver, kidney, spleen, and thymus were excised; the spleen and thymus were immediately weighed. The thymus and spleen indices were calculated according to the formula, index (mg/g) = (weight of thymus or spleen)/body weight.

2.4. Lymphocyte proliferation assay

The mouse spleens were aseptically removed from the sacrificed mice using scissors and forceps in 0.1 M cold PBS, gently homogenised, and passed through a 40 μm nylon cell strainer to obtain single-cell suspensions in accordance with the method used by Yuan, Song, Li, Li, and Dai (2006). The trythrocytes in the cell mixture were washed via hypo-osmostic haemolysis, and the cells were resuspended to a final density of 5×10^6 cells/ml in RPMI 1640 medium supplemented with 10% newborn bovine serum (Invitrogen Corp., Carlsbad, CA, USA), 100 U/ml streptomycin, and 100 U/ml penicillin. Spleen cells (100 μl/well) were seeded into a 96-well plate containing ConA (8 μg/ml). The spleen cells were then cultured for 3 days in 5% CO_2 atmosphere at 37 °C, and then further incubated for 4.5 h with 10 μl MTT (5 mg/ml) per well. The plate was centrifuged at 200 × *g* for 15 min, and the supernate was discarded. DMSO (100 μl) was added to each well, which was then shaken until all crystals dissolved. The absorbance at 570 nm was measured on a microplate reader (Thermo Multiskan MK3, USA).

2.5. Phagocytic index

The function of the macrophage cells was assessed via a carbon clearance test performed on three mice from each group according to the procedure of Wang et al. (2011). Each mouse was intravenously injected with diluted India ink at 100 μl/10 g body weight. Blood specimens were collected at 2 min (t_1) and 10 min (t_2) from the retinal venous plexuses, and 20 μl blood was then mixed with 2 ml 0.1% Na_2CO_3. The absorbance at 600 nm was measured on a UV-visible spectrophotometer with 0.1% Na_2CO_3 as the blank. The liver and the spleen were weighed, and the phagocytic index was calculated as follows:

$$K = \frac{lg\, OD_1 - lg\, OD_2}{t_2 - t_1}$$

where OD_1 was for t_1 and OD_2 was for t_2.

Phagocytic index $\alpha = \sqrt[3]{K} \times A/(B + C)$, where A is the body weight, B is the liver weight, and C is the spleen weight.

2.6. Biochemical assay

The organ homogenates (including the liver, heart, and kidney) were prepared in a 0.1 g/ml wet weight of ice-cold isotonic physiological saline. The samples were centrifuged at 2000 × *g* at 4 °C for 10 min, and the supernates were used for the measurement of the protein, T-AOC, MAD, CAT, SOD, and GSH-Px levels. The SOD activity and the MDA and TAOC levels were measured via spectrophotometric methods. The MDA level was detected using 2-thiobarbituric acid (Uchiyama & Mihara, 1978). The SOD activity was analysed via autooxidation of pyrogallol (Marland & Marklund, 1974). The TAOC level was measured using the ferric reducing/antioxidant power assay (Benzie & Strain, 1996). The enzyme activity was expressed in nanomoles per milligram of protein.

2.7. Statistical analysis

All data are presented as the mean ± SD, analysed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). The statistical analysis was evaluated via one-way ANOVA followed by Scheffe's test to detect the intergroup differences. A $P < 0.05$ values was considered statistically significant.

3. Results

3.1. Effect of CMP on mouse spleen and thymus indices

The spleen and thymus indices can reflect the immune function and prognosis of an organism. As shown in Fig. 1, the spleen and thymus indices of the model group remarkably decreased compared with those of the normal group ($P < 0.05$). CMP increased the spleen

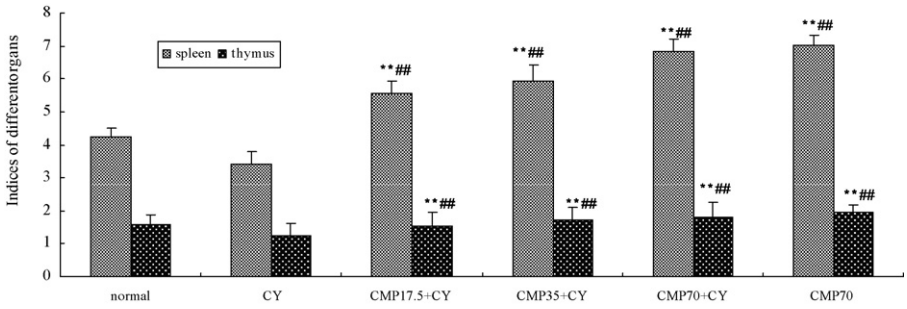

Fig. 1. Effects of CMP on the internal organ indices of the CY-induced mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

and thymus indices in the CY-treated mice in a dose-dependent manner at 17.5, 35, and 70 mg/kg, indicating that CMP can reverse the CY-induced atrophy of immune organs.

3.2. Effect of CMP on cellular immunity in mice

Spleen lymphocyte proliferation was examined to understand the mechanism of the immunoregulatory activity of CMP. As shown in Fig. 2, the spleen lymphocyte proliferation of the model group remarkably decreased compared with that of the normal group ($P<0.05$). CMP significantly increased the spleen lymphocyte proliferation in CY-treated mice in a dose-dependent manner at 17.5, 35, and 70 mg/kg compared with the model group, suggesting that CMP is directly mitogenic for mouse splenocytes.

3.3. Effect of CMP on the phagocytic activity of the macrophage system

Carbon clearance tests were performed to determine the effect of CMP on macrophage activation. The phagocytic index α of the model group was lower compared with that of the NS group (Fig. 3). CMP effectively increased the α value of the CY-treated mice in a dose-dependent manner. At the high CMP dose (70 mg/kg/d), the phagocytic activity was restored to above the normal level (from 4.51 to 4.74), demonstrating that CMP can enhance the macrophage function in CY-treated mice.

3.4. Antioxidant activity of CMP in vivo

3.4.1. Effect of CMP on the activity of SOD in the different organs of the immunosuppressed mice

Fig. 4 shows that CY significantly reduced the SOD activity ($P<0.01$) in the hearts, livers and kidneys compared to the NS control group. All CMP doses significantly increased the SOD activity relative to the model group ($P<0.01$).

3.4.2. Effect of CMP on the activity of CAT in the different organs of the immunosuppressed mice

Fig. 5 shows the marked reductions CAT activity ($P<0.01$) in the hearts, livers and kidneys of mice in the CY-treated and NS control groups. CMP (17.5, 35, and 70 mg/kg) significantly increased CAT activity compared to the model group ($P<0.01$).

3.4.3. Effect of CMP on the activity of GSH-Px in the different organs of the immunosuppressed mice

Fig. 6 shows the significant reductions in GSH-Px activity ($P<0.01$) in the hearts, livers and kidneys of the CY-treated and NS control groups. All CMP doses significantly increased the GSH-Px activity compared to the model group ($P<0.01$).

3.4.4. Effect of CMP on the activity of TAOC in the different organs of the immunosuppressed mice

Fig. 7 shows the remarkable reductions in TAOC activity ($P<0.01$) in the hearts, livers and kidneys of the CY-treated and NS control groups. CMP (17.5, 35, and 70 mg/kg) significantly increased the TAOC activity compared to the model group ($P<0.01$).

3.4.5. Effect of CMP on the activity of MDA in the different organs of the immunosuppressed mice

Fig. 8 shows the significant increases in MDA levels ($P<0.01$) in the hearts, livers and kidneys of the CY-treated and NS control groups. All CMP doses significantly decreased the MDA levels compared to the model group ($P<0.01$).

4. Discussions

CY is a cytotoxic chemotherapeutic drug that acts as an important agent in tumour treatment. However, its administration leads to immunosuppression, which may be life-threatening (Hong, Yan, & Baran, 2004). Traditional Chinese medications for immunosuppression treatment are available. In the present study, the protective effects of CMP in reversing the immunosuppression caused by CY treatment were investigated. The results indicate that CMP can reverse the CY-induced atrophy of immune organs.

In line with the usage of Cordyceps in China, Chinese medicines are strongly recommended for the ageing population to enhance their immune system and prevent possible infection. Immunostimulation itself is regarded as one of the important strategies to improve the body′s defense mechanism in elderly people as well as in cancer patients. A significant amount of experimental evidence suggests that polysaccharides from mushrooms enhance the host immune system by stimulating natural killer cells, T-cells, B-cells, and macrophage-dependent immune system responses (Dalmo & Boqwald, 2008; Dennert & Tucker, 1973). Polysaccharides obtained from different natural sources represent a structurally diverse class of macromolecules, which exert their antitumour action mostly by activating various immune system responses (Schepetkin & Quinn, 2006). In previous studies, Cordyceps polysaccharides were found to induce the functional activation of macrophages through the upregulation of cytokine expression (tumour necrosis factor alpha and IL-1β) and nitric oxide (NO) release (Lee et al., 2010), as well as the production of IL-6 and IL-10 in a dose-dependent manner. They promote the mRNA and protein expressions of inducible nitric oxide synthase, induce T-lymphocyte proliferation and the secretion of IL-2, IL-6, and IL-8, and increase the phagocytic and enzymatic activities of the acid phosphatase of macrophages. In the current study, the administration of CMP significantly enhanced the spleen lymphocyte proliferation and increased the phagocytic index α in a dose-dependent manner, thereby implying that CMP can also enhance the spleen lymphocyte activity and macrophage function in CY-treated mice.

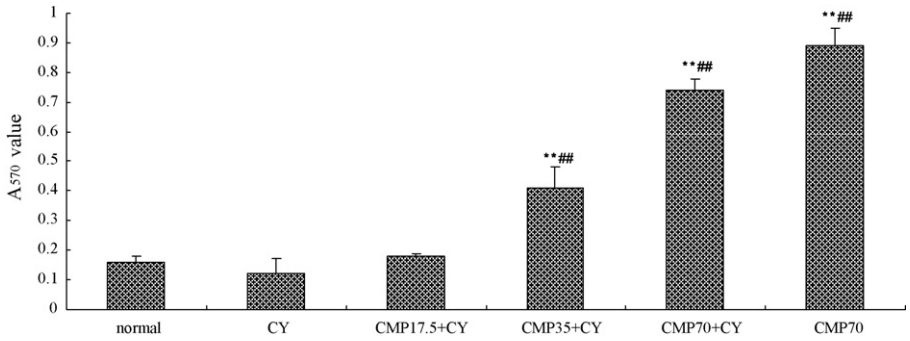

Fig. 2. Effect of CMP on the spleen lymphocyte proliferation in CY-treated mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

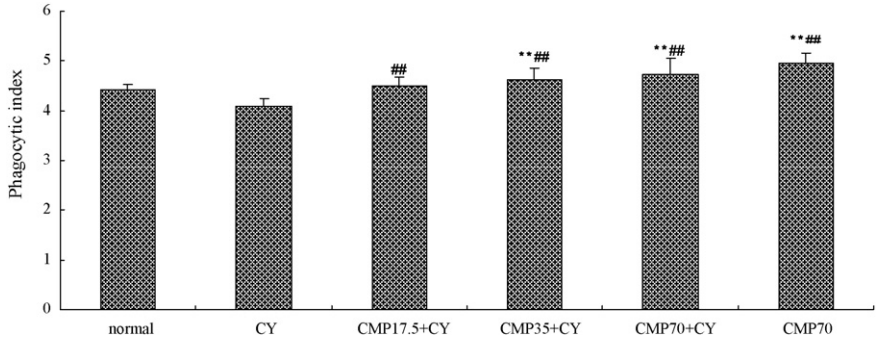

Fig. 3. Effect of CMP on the phagocytic index in the CY-treated mice. $^{*}P<0.05$,$^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

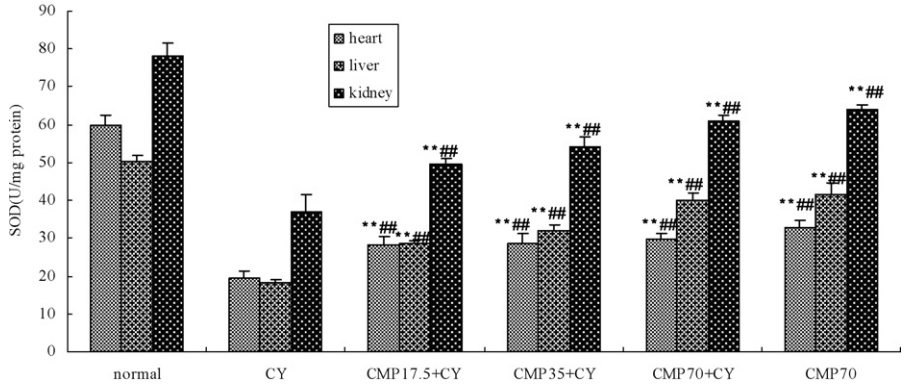

Fig. 4. Effect of CMP on the SOD activity in the hearts, livers and kidneys of the immunosuppressed mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

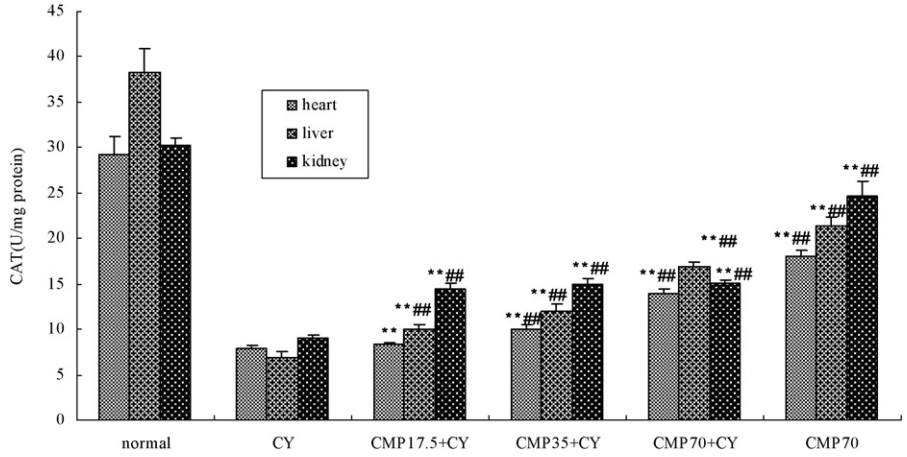

Fig. 5. Effect of CMP on the CAT activity in the hearts, livers and kidneys of the immunosuppressed mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

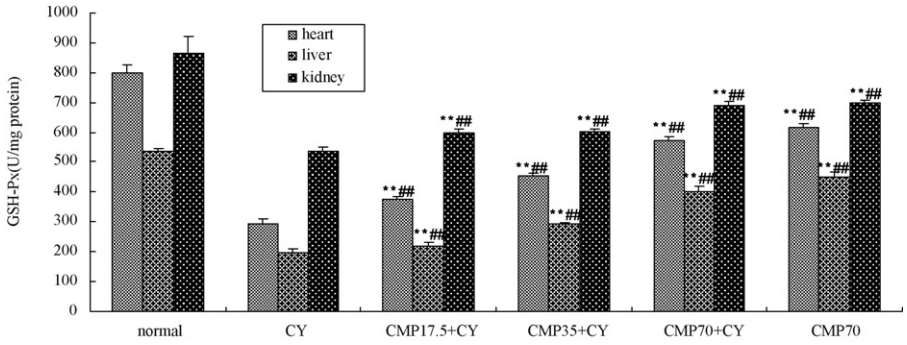

Fig. 6. Effect of CMP on the GSH-Px activity in the hearts, livers and kidneys of the immunosuppressed mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

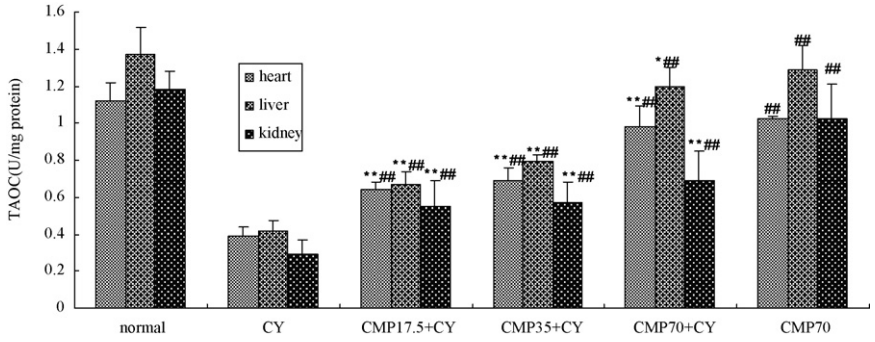

Fig. 7. Effect of CMP on the TAOC activity in the hearts, livers and kidneys of the immunosuppressed mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

Free-radical-induced lipid peroxidation has been associated with a number of diseases. The excessive production of free radicals such as superoxide, hydroxyl radicals, hydrogen peroxide, and NO (collectively referred to as ROS) plays multiple important roles in tissue damage and loss of function in a number of tissues and organs (Simic, Bergtold, & Karam, 1989; Zheng & Huang, 2001). An increasing amount of evidence indicates that many kinds of polysaccharides have potential and potent capabilities of preventing oxidative damage in living organisms from free radical scavenging (Liu, Ooi, & Chang, 1997; Peterszegi, Robert, & Robert, 2003; Zhang et al., 2003). Cordyceps polysaccharides can scavenge free radicals, and the antioxidant activity of *C. militaris* was even stronger than that of the *C. sinensis* and *Cordyceps kyushuensis* (Chen, Luo, Li, Sun, & Zhang, 2004). The polysaccharide fractions P70-1 and CBP-1 from *C. militaris* showed hydroxyl radical-scavenging activities in a concentration-dependent manner, with IC50 values of 0.548 and 0.638 mg/ml in vitro (Yu et al., 2007, 2009). The result of the present study is consistent with that of P70-1 and CBP-1 in vitro. When the mice were treated with CY, the T-AOC, CAT, SOD, and GSH-Px levels in the heart, liver, and kidney remarkably decreased and the MDA levels clearly increased. However, the administration of CMP (17.5, 35, and 70 mg/kg) can cause significant increases in the SOD, CAT, GSH-Px, and TAOC levels as well as a decrease in the MDA levels, thereby indicating that CMP can be effective in scavenging various types of oxygen free radicals and their products.

In conclusion, the current study demonstrates that CMP alone improved the immune functions and exhibited effective antioxidant activities in the CY-treated mice. CMP should be explored as a good immunomodulatory agent with antioxidant activity and may be applied to antineoplastic immunotherapy in combination with chemotherapeutic agents. However, further investigation on the

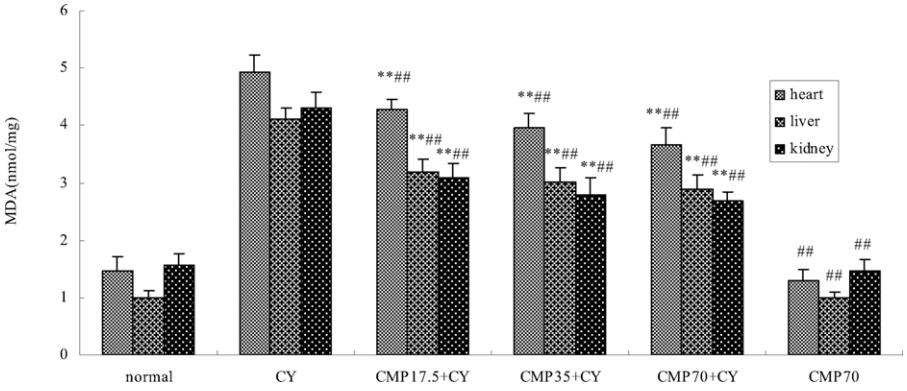

Fig. 8. Effect of CMP on the MDA level in the hearts, livers and kidneys of the immunosuppressed mice. $^{*}P<0.05$, $^{**}P<0.01$ compared with the NS group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the model group. Values are means ± SD.

mechanisms underlying free radical scavenging of CMP is necessary.

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References

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, the FRAP assay. *Analytical Biochemistry*, *239*, 70–76.

Chen, C., Luo, S. S., Li, Y., Sun, Y. J., & Zhang, C. K. (2004). Study on antioxidant activity of three Cordyceps sp. by chemiluminescence. *Shanghai Journal of Traditional Chinese Medicine*, *38*(7), 53–55.

Chen, W. X., Zhang, W. Y., Shen, W. B., & Wang, K. C. (2010). Effects of the acid polysaccharide fraction isolated from a cultivated *Cordyceps sinensis* on macrophages in vitro. *Cellular Immunology*, *262*, 69–74.

Cheung, J. K., Li, J., Cheung, A. W., Zhu, Y., Zheng, K. Y., Bi, C. W., et al. (2009). Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. *Journal of Ethnopharmacology*, *124*(1), 61–68.

Dalmo, R. A., & Boqwald, J. (2008). Beta-glucans as conductors of immune symphonies. *Fish Shellfish Immunology*, *25*, 384–396.

Dennert, G., & Tucker, D. (1973). Antitumor polysaccharide lentinan A T cell adjuvant. *Journal of the National Cancer Institute*, *51*, 1727–1729.

Gai, G. Z., Jin, S. J., Wang, B., Li, Y. Q., & Li, C. X. (2004). The efficacy of *Cordyceps militaris* capsules in treatment of chronic bronchitis in comparison with Jinshuibao capsules. *Chinese Journal of New Drugs*, *13*, 169–171.

Hong, F., Yan, J., & Baran, J. T. (2004). Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. *Journal of Immunology*, *173*, 797–806.

Kim, C. S., Lee, S. Y., Cho, S. H., Ko, Y. M., Kim, B. H., Kim, H. J., et al. (2008). *Cordyceps militaris* induces the IL-18 expression via its promoter activation for IFN-γ production. *Journal of Ethnopharmacology*, *120*(3), 366–371.

Lee, J. S., Kwon, J. S., Yun, J. S., Pahk, J. W., Shin, W. C., Lee, S. Y., et al. (2010). Structural characterization of immunostimulating polysaccharide from cultured mycelia of *Cordyceps militaris*. *Carbohydrate Polymers*, *80*(4), 1011–1017.

Li, S. P., Li, P., Dong, T. T. X., & Tsim, K. W. K. (2001). Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps mycelia*. *Phytomedicine*, *8*(3), 207–212.

Li, S. P., Yang, F. Q., & Tsim, K. W. (2006). Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine. *Journal of Pharmaceutical and Biomedical Analysis*, *41*, 1571–1584.

Li, S. P., Zhao, K. J., Ji, Z. N., Song, Z. H., Dong, T. T. X., Lo, C. K., et al. (2003). A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. *Life Sciences*, *73*, 2503–2513.

Liu, F., Ooi, V. E., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*, *60*, 763–771.

Marland, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, *47*, 469–474.

Novak, M., & Vetvicka, V. (2008). Beta-glucans, history, and the present: Immunomodulatory aspects and mechanisms of action. *Journal of Immunotoxicology*, *5*, 47–57.

Park, S. E., Kim, J. G., Lee, Y. W., Yoo, H. S., & Cho, C. K. (2009). Antitumor activity of water extracts from *Cordyceps militaris* in NCI-H460 cell xenografted nude mice. *Journal of Acupuncture and Meridian Studies*, *2*, 294–300.

Paterson, R. R. M. (2008). Cordyceps-A traditional Chinese medicine and another fungal therapeutic biofactory? *Phytochemistry*, *69*, 1469–1495.

Peterszegi, G., Robert, A. M., & Robert, L. (2003). Protection by L-fucose and fucose-rich polysaccharides against ROS-produced cell death in presence of ascorbate. *Biomedicine & Pharmacotherapy*, *57*, 130–133.

Rao, Y. K., Fang, S. H., Wu, W. S., & Tzeng, Y. M. (2010). Constituents isolated from *Cordyceps militaris* suppress enhanced inflammatory mediator's production and human cancer cell proliferation. *Journal of Ethnopharmacology*, *131*(2), 363–367.

Schepetkin, I. A., & Quinn, M. T. (2006). Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, *6*, 317–333.

Simic, M. G., Bergtold, D. S., & Karam, L. R. (1989). Generation of oxygen radicals in biosystems. *Mutation Research*, *214*, 3–12.

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, *86*, 271–278.

Wang, H., Wang, M. Y., Chen, J., Tang, Y., Dou, J., Yu, J., et al. (2011). A polysaccharide from *Strongylocentrotus nudus* eggs protects against myelosuppression and immunosuppression in cyclophosphamide-treated mice. *International Immunopharmacology*, *11*(11), 1946–1953.

Yu, R. M., Yang, W., Song, L. Y., Yan, C. Y., Zhang, Z., & Zhao, Y. (2007). Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*. *Carbohydrate Polymers*, *70*(4), 430–436.

Yu, R. M., Yin, Y., Yang, W., Ma, W. L., Yang, L., Chen, X. J., et al. (2009). Structural elucidation and biological activity of a novel polysaccharide by alkaline extraction from cultured *Cordyceps militaris*. *Carbohydrate Polymers*, *75*(1), 166–171.

Yuan, H., Song, J., Li, X., Li, N., & Dai, J. (2006). Immunomodulation and antitumor activity of kappa-carrageenan oligosaccharides. *Cancer Letters*, *243*, 228–234.

Zhang, Q. B., Li, N., Zhou, G. F., Lu, X. L., Xu, Z. H., & Li, Z. (2003). In vivo antioxidant activity of polysaccharide fraction from *Porphyra haitanesis* (Rhodephyta) in aging mice. *Pharmacological Research*, *48*, 151–155.

Zheng, H. C., & Cai, S. Q. (2004). *Medicinal botany and pharmacognosy*. Beijing: People's Medical Publishing House.

Zheng, R. L., & Huang, Z. Y. (2001). *Reactive oxygen species*. Beijing: China Higher Education Press/Springer Press.