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Full Length Research Paper

Immune stimulating activity of water-soluble polysaccharide fractions from *Dendrobium nobile* Lindl.

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Four water-soluble polysaccharides (DNP1-1, DNP2-1, DNP3-1 and DNP4-2) were obtained from the aqueous extracts of the stems of *Dendrobium nobile* Lindl. by hot water extraction, ethanol precipitation, and fractionated by DEAE-cellulose ion exchange chromatography and Sephadex G-200 gel filtration chromatography. The evaluation of tumor inhibition and immunomodulatory activity suggested that DNP4-2 could significantly increase the immune index, and strongly promote the secretion of IL-2, TNF-α and IFN-γ, and also decrease the concentrations of MDA in blood serum. DNP4-2 should be explored as a potential anti-tumor drug.

Key words: *Dendrobium nobile* Lindl., immune stimulating, polysaccharide.

INTRODUCTION

Dendrobium nobile Lindl. (Chinese name "Jin Chai Shi Hu") is one of the most famous Dendrobium plants in traditional Chinese medicine, it belongs to Orchidaceae, is a precious herbal plant in Chinese traditional medicine as a therapeutic for nourishing the stomach, promote secretion of saliva, and reduce fever (Shu et al., 2004), and it is one of the five species which were specified in the Chinese Phamacopeia (2005). To elucidate the pharmacological mechanism of *D. nobile* Lindl. much research has been carried out on the low molecular compounds, such as bibenzyls (Zhang et al., 2006), alkaloids (Liu and Zhao, 2003) and phenanthrenes (Lee et al., 1995; Yang et al., 2007; Wang et al.,1985). As for the compounds of polysaccharides from *D. nobile* Lindl. little information was obtained (Luo et al., 2009; Wang et al., 2010). Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects, which is a major problem associated with immunomodulatory and antioxidant polysaccharides (Luo et al., 2011; Fan et al., 2009). Thus, plant polysaccharides are ideal candidates for therapeutics with immunomodulatory and antitumor effects and low toxicity (Schepetkin et al., 2006). Polysaccharides from some Dendrobium species exhibited high immunomodulatory and antitumor activities, such as *Dendrobium huoshanense* (Zha et al., 2007; Yves et al., 2008) and *Dendrobium denneanum* (Fan et al., 2010, 2011). Although some polysaccharides fractions from *D. nobile* Lindl. has strong anti-tumor activities (Wang et al., 2010), the mechanism of antitumor activity and immunomodulation effect of polysaccharides from *D. nobile* Lindl. were not reported. Therefore, the aim of present study is to evaluate the mechanism of antitumor activities and immunomodulation effects of four polysaccharide fractions from *D. nobile* Lindl *in vivo*.

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MATERIALS AND METHODS

Drugs and reagents

Cyclophosphamide (CTX) was purchased from Hengrui medicine co. the tumor necrosis factoralpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-2 (IL-2) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Senxiong Biotech Co. T-series Dextran and Sephadex G-200 were purchased from Pharmacia Co. (Uppsala, Sweden). The standard monosaccharides (glucose, mannose, rhamnose, galactose, xylose and arabinose) were purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All the other chemicals and reagents were of grade AR.

Preparation for polysaccharides

D. nobile Lindl. polysaccharides were isolated and purified as described previously (Luo et al., 2010). Four major polysaccharide

peaks, DNP1-1, DNP2-1, DNP3-1, and DNP4-2 were collected and lyophilized. And the average molecular weights (Mws) of these fractions were 136, 27.7, 11.8 and 11.4 kDa, respectively. Monosaccharide components analysis indicated that DNP1-1 was composed of rhamnose: arabinose: xylose: mannose: glucose: galactose =2.18: 4.00: 1.00: 12.12: 46.10: 33.72. DNP2-1 was composed of only three monosaccharides, namely mannose: glucose: galactose=1.00: 3.44: 1.75. DNP3-1 was composed of five monosaccharides, and rhamnose: arabinose: mannose: glucose: galactose =1.00: 2.47: 1.59: 3.35: 16.65. DNP4-2 was composed of five monosaccharides: rhamnose, arabinose, mannose, glucose, galactose in a molar ratio of 2.74: 1.00: 2.31: 5.11:9.54.

In vivo anti-tumor test

Animals and treatment

Kunming mice between 6 and 8 weeks old (weight: 20.0±2.0 g) were purchased from the Experimental Animal Center of Sichuan Academy of Medical Sciences (Chengdu, China). The mice were kept in separated cages at a temperature of 21 ± 1°C and a 50 to 60% of relative humidity. They underwent 12 h light-and-dark cycles with free access to food and water. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. A total of 150 mice were evenly and randomly divided into fifteen groups including a normal control group, a positive control group (Cyclophosphamide, 1.25 mg/ml), a S180 model control group (normal saline) and the polysaccharide groups (5, 2.5 and 1.25 mg/ml). Sarcoma 180 cells were generously donated by Dr. Yu of Sichuan Academy of Chinese Medicine Sciences. Sarcoma 180 cells were passed into mice ascites. Then, ascites was inoculated subcutaneously 0.2 ml (5.0 x 10^7 cells/ml) into the sword arm of each experimental mouse. Normal control mice were not inoculated Sarcoma 180. All the groups were administered daily by intraperitoneal injection (0.2 ml).

Tumor inhibition effect

The tested samples (5, 2.5 and 1.25 mg/ml of each polysaccharide) and CTX (1.25 mg/mlt) were dissolved in saline, and then injected intraperitoneally (i.p.) once a day for 10 days, starting 24 h after tumor inoculation. The normal control and model control mice received an equal volume of saline (0.2 ml). All animals were weighted and sacrificed after 24 h when the last tested had been administered. The inhibitory rate was calculated as [(A–B)/A] x 100% (Furukawa et al., 2000), where A was the tumor weights of the model group, and B was the tumor weights of the tested group.

Analysis of immune index

After these mice were sacrificed by cervical dislocation, the spleens and thymus of these mice were recovered by anatomized. The spleen index was determined from the weight of the spleen and the results are expressed as the formula: weight of spleen (mg)/ body weight (g). The thymus index was measured by using the same method.

Determination of IL-2, TNF-α, and IFN-γ by the ELISA method

The blood samples were obtained from orbitae of these mice and the serum was collected after the blood samples had been processed by centrifugation at 2500 rpm for 10 min at 10°C (Ruan et al., 2005). The interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) concentration were measured with an enzyme-linked immunosorbent assay (ELISA kit, Shanghai Senxiong Biotech) according to the indication of the manufacturer.

Analysis of SOD and MDA

Twenty-four hours after the last drug administration, blood samples were obtained from the eye pit of the mice and processed for serum (Luo et al., 2011). The superoxide dismutase (SOD) activity and the malondialdehyde (MDA) level were also measured. SOD activity (U/ml) was tested with the SOD assay kit.

Statistical analysis

The data were expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the results were processed by SPSS software.

RESULTS AND DISCUSSION

In vivo tumor inhibition effect of the polysaccharide fractions

Although any reduction of the tumor volume was not the only standard of anti-tumor, the tumor masses reduction often indicate the strong anti-tumor effect. So, tumor inhibition rate was usually used as an index of screening the anti-tumor drug. Cyclophosphamide is generally used for treatment of various types of cancers. It is a clinically approved anticancer agent that works by slowing or stopping cell growth. The tumor inhibition rates of different polysaccharide fractions and CTX were shown in Figure 1. The results indicated that the positive control exhibited significant anti-tumor activity with a high tumor inhibition rate in all groups. Meanwhile, different doses of four polysaccharide fractions exhibited different tumor inhibition activities. The effects of all the polysaccharide samples were not in a concentration-dependent manner. In the four polysaccharide fractions, DNP4-2 exhibited strongest tumor inhibition activities. Especially at the dose of 2.5 mg/ml, the anti-tumor activity was 67.01%, followed by 5 and 1.25 mg/ml. On the contrary, the antitumor activities of the other polysaccharide fractions were weak. So, the results above proved that the water-soluble polysaccharide with 11.4 kDa (DNP4-2) has significant tumor inhibition effect.

Analysis of immune index

To evaluate the effect of four polysaccharide fractions on the immune system, the spleen and thymus index were calculated. The effects of different samples on thymus index were showed in Figure 2, from the figure, the value of thymus index in the CTX group was very low, which proved that the toxicity of CTX on the mice. On the contrary, all polysaccharide fractions exhibited strong increasing effects on the thymus index. Especially for DDP4-2, at the dose of 2.5 mg/ml, significant increasing

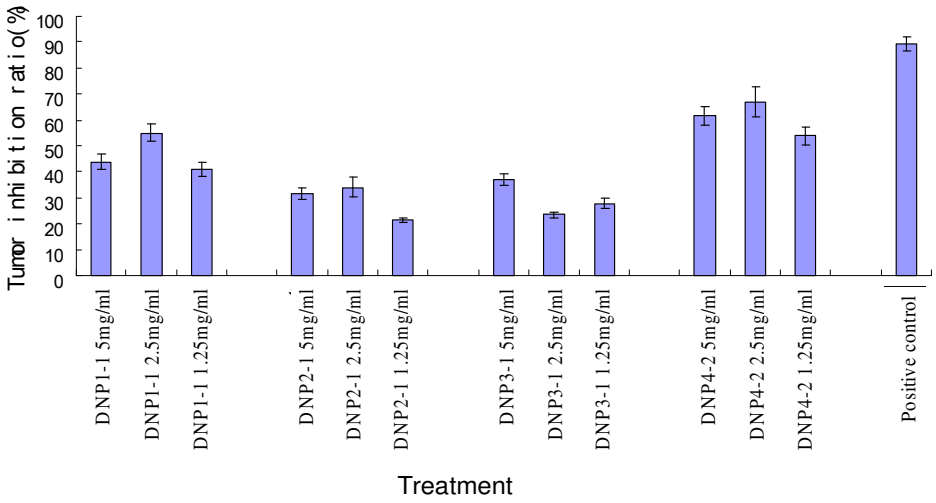

Figure 1. Tumor inhibitory rate was calculated as [(A–B)/A] × 100%, where A was the tumor weights of the model group, and B was the tumor weights of the tested group. Values of tumor inhibitory rate are means ± S.D, (n = 10).

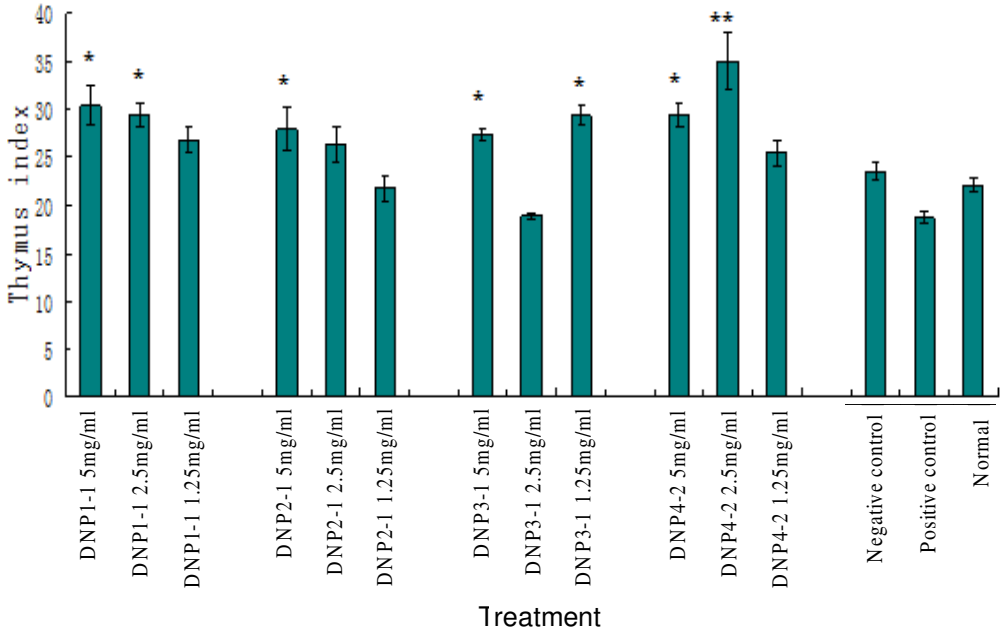

Figure 2. Thymus index was measured in the ratio of thymus. Values are means ± S.D, (n=10). *P<0.05, **P<0.01 vs. Negative control.

was observed compared with the negative control, but not in a concentration-dependent manner. At the same time, DNP1-1 with 2.5 and 5 mg/ml, DNP4-2 with 5 mg/ml, DNP3-1 with 5 and 1.25 mg/ml also exhibited high increase in the thymus index.

The results of determination of spleen index showed in Figure 3. All the polysaccharide-treatment groups except DNP4-2 exhibited lower value of spleen index, which indicated that there was no significant increasing effect of the three polysaccharides on spleen index in the mice. There was significant decreasing effect on the CTX group compared with the negative control. However, at the dose of 2.5 mg/ml of DNP4-2, significant increasing was observed compared with the negative control, but not in a concentration-dependent manner. Therefore, the results above indicated that the polysaccharide fraction DNP4-2 could significantly increase the immune system of S180 mice, which suggested that immunomodulationmay be the mechanism of the tumor inhibition activity of 2.5 mg/ml DNP4-2.

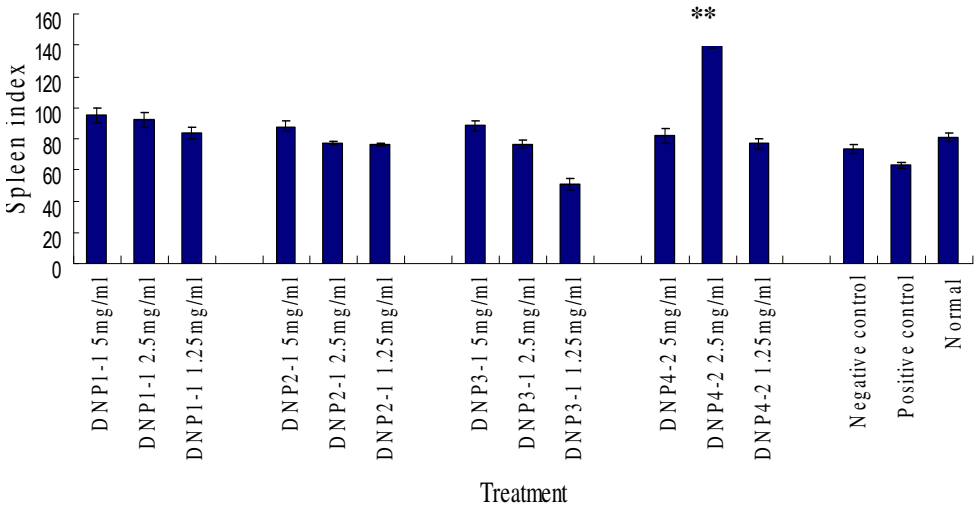

Figure 3. Spleen index was measured in the ratio of spleen. Values are means ± S.D, (n = 10). *P<0.05, **P<0.01 vs. Negative control.

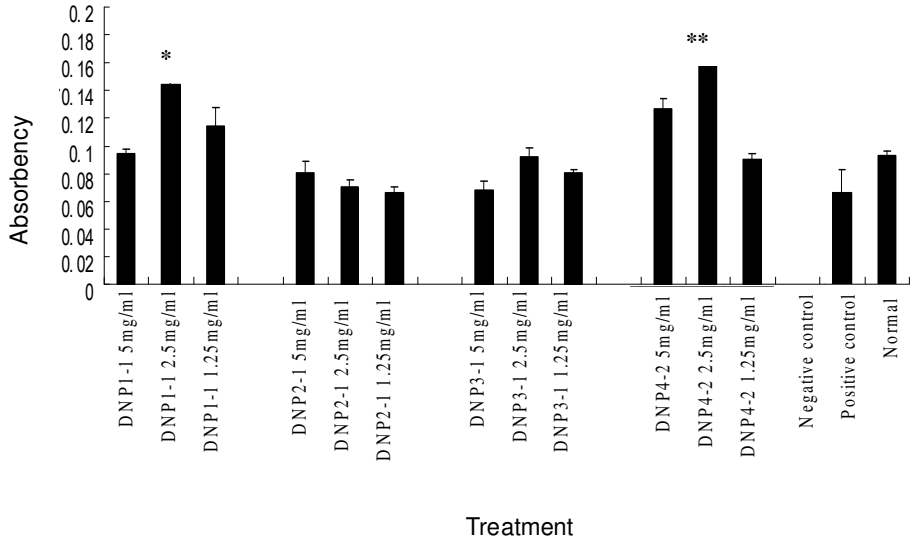

Figure 4. Effects of different polysaccharides and CTX on the secretion of IL-2. P<0.05, **P<0.01 vs. negative control.

Determination of IL-2, TNF-α and IFN-γ by the ELISA method

Serum from each groups were collected after administration of the drugs. The interleukin-2, tumor necrosis factor-alpha and interferon-gamma concentration were measured with an enzyme-linked immunosorbent assay according to the indication of the manufacturer. IFN-γ is an important immunoregulatory molecule. It induces the generation of T cells, activates macrophages, and regulates crossly Th1 and Th2 cells. TNF-α and IFN-γ can enhance immunoregulatory ability each other towards tumor.

From the results of Figures 4, 5 and 6, the concentrations of TNF-α, IFN-γ and IL-2 in the group with CTX-treated were significantly low, which indicated CTX could not promote the secretion of the three cytokines. However, the concentrations of TNF-α, IFN-γ and IL-2 with DNP4-2-treated have different degrees increase. Especially at the dose of 2.5 mg/ml, DNP4-2 have strongly promoted the secretion of the three cytokines (P<0.01). Therefore, the results indicated that the level of three cytokines were significant increase compared with the control, that was 2.5 mg/ml DNP4-2-treated could

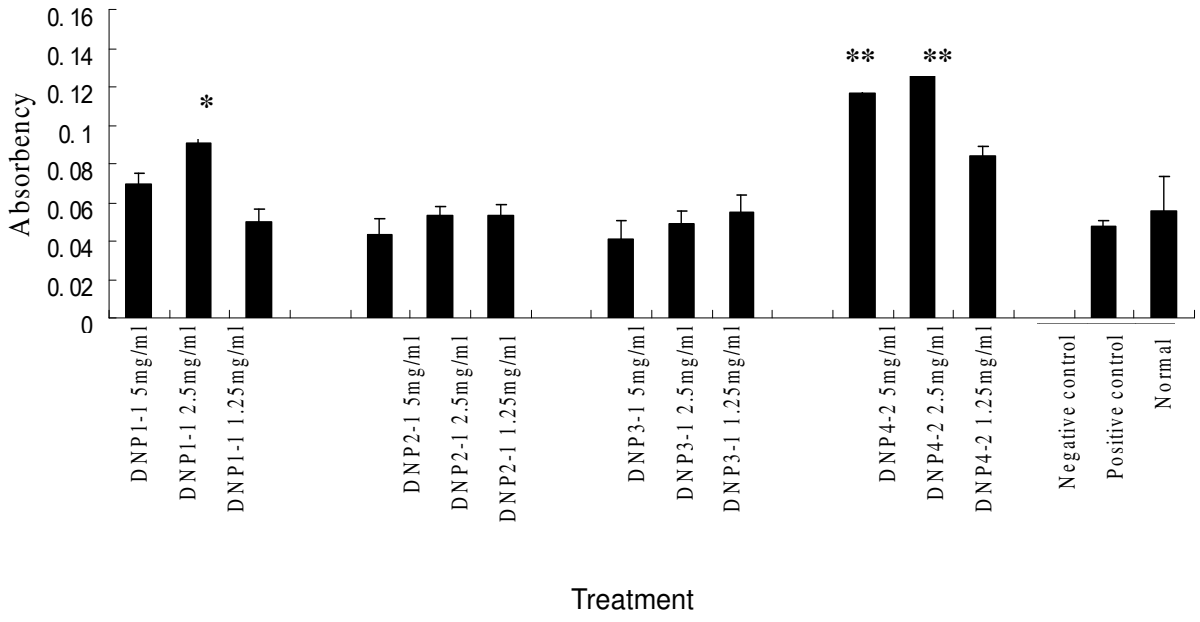

Figure 5. Effects of different polysaccharides and CTX on the secretion of TNF-α. *P<0.05, **P<0.01 vs. negative control.

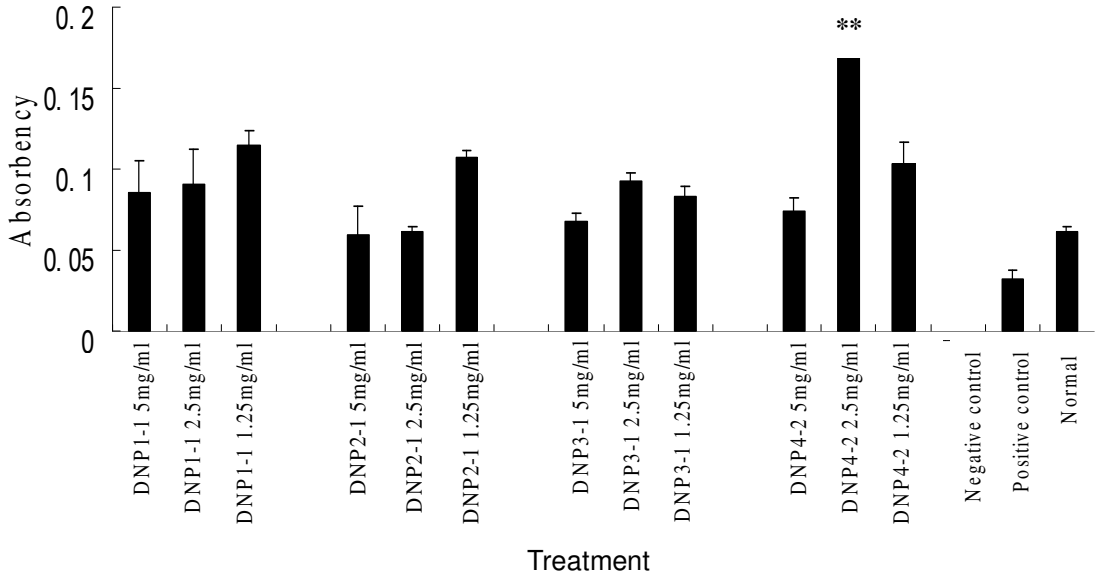

Figure 6. Effects of different polysaccharides and CTX on the secretion of IFN-γ. *P<0.05, **P<0.01 vs. negative control.

augment well IL-2, TNF-α and IFN-γ production.

Analysis of SOD and MDA

There are published reports demonstrated that cancer is related to the increase of radicals *in vivo* (Lan and Kang, 2002). Therefore, the aim of determine of scavenging effect on radical is to estimate the relation on radical scavenging ability and anti-tumor activity in our experiment. SOD activity (U/ml) was tested with the SOD assay kit. Superoxide was generated in xanthine oxidase and hypoxanthine, and the superoxide scavenging effect of serum was determined according to the method of Oyanagui et al. (1984). SOD activity of the serum was expressed in U/ml of the sample. As shown in Figure 7, the SOD activity in the negative control was very poor, which indicated that the ability of antioxidant system in

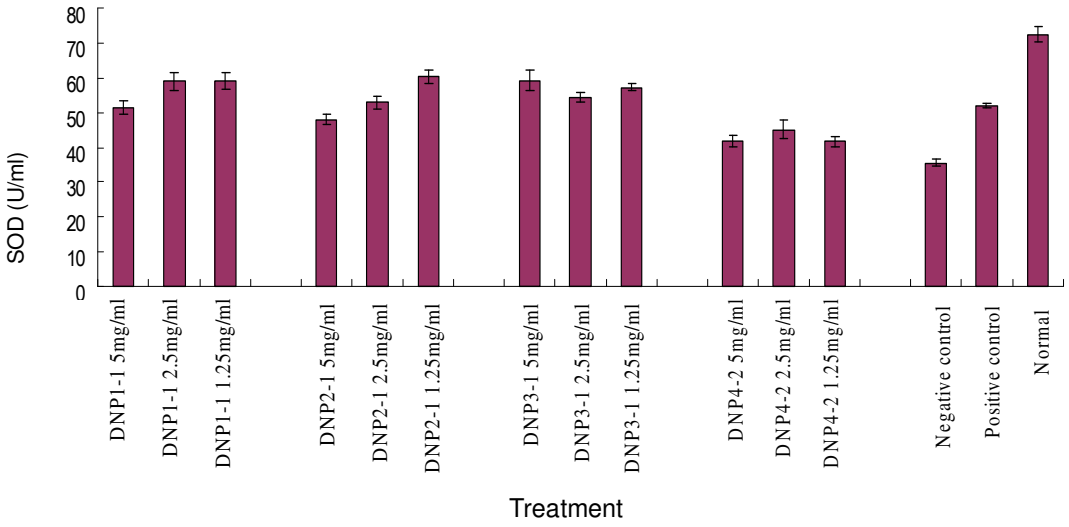

Figure 7. The scavenging effect of polysaccharides fractions on SOD. *P<0.05, **P<0.01 vs. normal control.

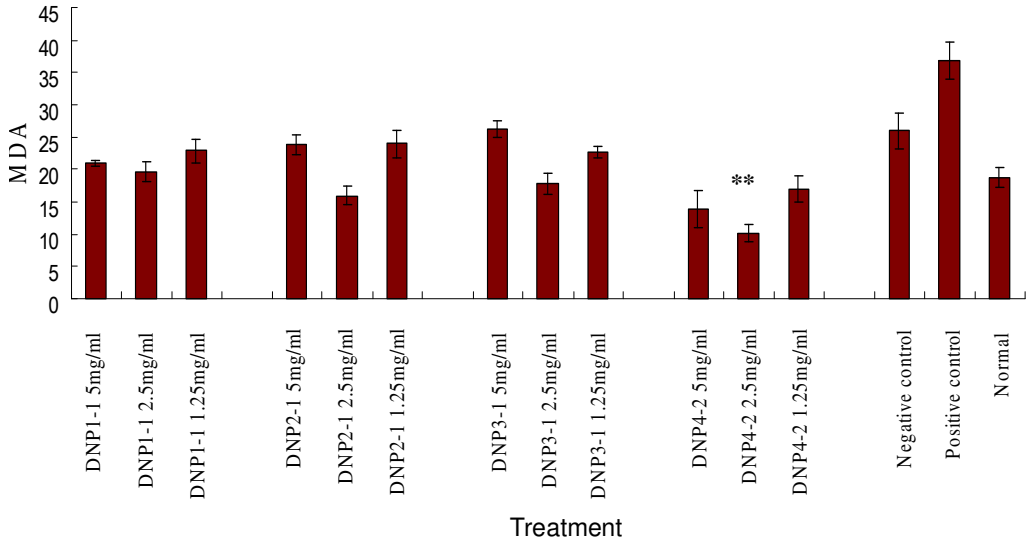

Figure 8. The scavenging effect of polysaccharides fractions on MDA. *P<0.05, **P<0.01 vs. negative control.

the mice would be decreased when tumor inoculation. At the same time, SOD activities of all fractions and CTX groups were lower than that of the normal control group. The results suggested that these polysaccharides fractions from *D. nobile* Lindl. could not increasing the SOD activities in S180 mice.

MDA is formed during oxidative degeneration as a product of free oxygen radicals (Valenzuela et al., 1990), which is accepted as an indicator of lipid peroxidation (Neilsen et al., 1997). MDA was reported to be higher in cancer tissues than in non-diseased organ (Yagi et al., 1987). It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells (Jiau and Larry, 1977). Antitumor activity of these antioxidants is either through induction of apoptosis or by inhibition of neovascularization (Ming et al., 1998). The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor (Yerra et al., 2005). The concentrations of MDA in blood serum from the mice were determined with an MDA assay kit. The MDA value was estimated according to the thiobarbituric acid (TBA) method (Asakawa et al., 1980). The samples added with TBA were heated in an acidic environment. The absorbance of the resulting solution was measured at 532 nm. The results in Figure 8 exhibited a significant pattern of a decreasing MDA

concentration in all fractions groups. At 2.5 mg/ml, the concentration of MDA of DNP4-2 group was the lowest than that of all groups. This can be interpreted as a significant effect of DNP4-2 on MDA scavenging S180 mice.

Conclusions

In this work, it was concluded that DNP4-2 exhibited strong anti-tumor ability *in vivo*. Especially, at the dose of 2.5 mg/ml, DNP4-2 showed high anti-tumor rate (67.01%), which closed to the positive control. Meanwhile, DNP4-2 significant increased immune index of S180 mice, and strongly promoted the secretion of IL-2, TNF-α and IFN-γ. IL-2 could promote the long-term proliferation of T cells, TNF-α and IFN-γ can enhance immunoregulatory ability each other towards tumor. Therefore, enhancement of immunoregulatory ability was assumed to be the possible mechanism of DNP4-2 on inhibition of tumor. In addition, DNP4-2 has strong ability of reducing the concentrations of MDA in blood serum from the mice, which suggested that one possible mechanism of DNP4-2 on inhibition of tumor is preventing the lipid peroxidation *in vivo*. Further investigation on mechanism of DNP4-2 antitumor activities *in vivo* will be carried out in our later work. And with the results above, DNP4-2 could be explored as a potential antitumor drug.

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REFERENCES

Asakawa T, Matsuhita S (1980). Colouring conditions of Thiobarbituric acid test for detecting lipid hydroperoxides. Lipids, 15: 137-140.

Fan YJ, Chun Z, Luo AX, Luo AS, He T, He XJ (2010). *In vivo* Immunomodulatory Activities of Neutral Polysaccharide (DDP1-1) from *Dendrobium denneanum*. Chin J. Appl. Environ. Biol., 16(3): 376-379.

Fan YJ, He XJ, Zhou SD, Luo AX, He Tao, Chun Z (2009). Composition analysis and antioxidant activity of polysaccharide from *Dendrobium denneanum*. Int. J. Biol. Macromol., 45: 169-173.

Fan YJ, Luo AX (2011). Evaluation of anti-tumor activity of water-soluble polysaccharides from *Dendrobium denneanum*. Afr. J. Pharm. Pharmacol., 5(3).

Furukawa T, Kubota T, Tanino H, Oura S, Yuasa S, Murate H, Morita K, Kozakai K, Yano T, Hoffman RM (2000). Chemosensitivity of breast cancer lymph node metastasis compared to the primary tumor from individual patients tested in the histoculture drug response assay. Anticancer Res., 20: 3657-3658.

Jiau-Jian L, Larry WO (1977). Over expression of manganese-containing superoxide dismutase confers resistance to the cytotoxicity of tumor necrosis factor and/or hyperthermia. Cancer Res., 57: 1991-1998.

Lan K, Kang GG (2002). Free radical and tumor. Lab. Med. Clin. Tonsu., 3(1): 38-39.

Liu QF, Zhao WM (2003). A New Dendrobine-Type Alkaloid from *Dendrobium nobile*. Chinese Chem. Lett., 14(3): 278- 279.

Lee YH, Park JD, Baek NI, Kim SI, Ahn BZ (1995). *In vitro* and *in vivo* antitumoral phenanthrenes from the aerial parts of *Dendrobium nobile*. Planta. Med., 61(2): 178-180

Luo AX Ge ZF, Fan YJ, Luo AS, Chun Z, He XJ (2011). *In vitro* and *in vivo* Antioxidant Activity of a Water-Soluble Polysaccharide from *Dendrobium denneanum*. Mole., 16: 1579-1592.

Luo AX, Fan YJ, Luo AS (2011). *In vitro* Free Radicals Scavenging Activities of Polysaccharide from Polygonum Multiflorum Thunb. J. Med. Plants Res., 5(6): 966-972.

Luo AX, He XJ, Zhou SD, Fan YJ, He T, Chun Z (2009). *In vitro* antioxidant activities of a water-soluble polysaccharide derived from *Dendrobium nobile* Lindl. Extracts. Int. J. Biol. Macromol., 45: 359-363.

Luo AX, He XJ, Zhou SD, FanYJ, Luo AX, Chun Z (2010). Purification, composition analysis and antioxidant activity of the polysaccharides from *Dendrobium nobile* Lindl. Carbohyd. Polym., 79(4): 1014-1019.

Ming L, Jill CP, Jingfang JN, Edward C, Brash E (1998). Antioxidant action via p53 mediated apoptosis. Cancer Res., 58: 1723-1729.

Neilsen F, Mikkelsen BB, Neilsen JB, Andersen HR, Grandjean P (1997). Plasma malondialdehyde as biomar reference interval and effects of life-style factors. Clin. Chem., 47: 1209-1214.

Oyanagui Y (1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. Anal. Biochem., 142: 290-301.

Ruan Z, Su J, Dai HC, Wu MC (2005). Characterization and immunomodulating activities of polysaccharide from *Lentinus edodes*. Int. Immunopharmacol., 5: 811- 820.

Shu Y, Guo SX, Chen XM, Wang CL, Yang JS (2004). On the chemical constituents of *Dendrobium nobil*, Chin. Pharm. J., 6: 421-422.

Schepetkin IA, Quinn MT (2006). Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. Int. Immunopharmacol., 6: 317-333.

Valenzuela A (1990). The biological significance of determination in the assessment of tissue oxidative stress. Life Sci., 48: 301-309.

Wang HK, Zhao TF, Che CT (1985). Dendrobine and 3-Hydroxy-2-oxodendrobine from *Dendrobium nobile*. J. Nat. Prod., 48(5): 796-801.

Wang JH, Luo JP, Zha XQ (2010). Structural features of a pectic polysaccharide from the stems of *Dendrobium nobile* Lindl. Carbohyd. Polym., 81: 1-7.

Wang JH, Luo JP, Zha XQ, Feng BJ (2010). Comparison of antitumor activities of different polysaccharide fractions from the stems of *Dendrobium nobile* Lindl. Carbohyd. Polym., 79(1): 114.

Yves SYH, Cheng C, Sylvian KSL, Shih-Fen L, Wei-Ting H, Wen-Bin Y, Chih-Chien L, Ting-Jen RC, Chia-Chuan C, Jim-Min F, Chi-Huey W (2008). Structure and bioactivity of the polysaccharides in medicinal plant *Dendrobium huoshanense* .Bioorg. Med. Chem., 16: 6054-6068.

Yang HY, Sung SH, Kim YC (2007). Antifibrotic Phenanthrenes of *Dendrobium nobile* Stems. J. Nat. Prod., 70 (12): 1925-1929.

Yagi K (1987). Lipid peroxides and human diseases. Chem. Phys. Lipids, 45: 337-351.

Yerra R, Malaya G, Upal K M (2005). Antitumor Activity and in vivo Antioxidant Status of *Mucuna pruriens* (Fabaceae) Seeds against Ehrlich Ascites Carcinoma in Swiss Albino Mice. Iran J. Pharmacol. Ther., 4: 46-53.

Zhang X, Gao H, Wang NL, Yao XS (2006). Three new bibenzyl derivatives from Dendrobium nobile. J. Asian Nat. Prod. Res., 8(1-2): 113-118.

Zha XQ, Luo JP, Luo SZ, Jiang ST (2007). Structure identification of a new immunostimulating polysaccharide from the stems of *Dendrobium huoshanense*. Carbohyd. Res., 69: 86-93.