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Isolation and antitumor activities of acidic polysaccharide from *Gynostemma pentaphyllum* Makino

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ABSTRACT

Two acidic polysaccharides (GP-B1 and GP-C1) were obtained from *Gynostemma pentaphyllum*. The molecular weights (Mw) of the two fractions were 79 kDa for GP-B1 and 126 kDa for GP-C1. GP-B1 was composed of Gal, Ara, Man, Rha, Xyl, Glc, GalA and GlcA in a molar ration of 3.5:3.2:0.6:0.9:0.3:0.5:0.6:0.4. GP-C1 consisted of Gal, Ara, Man, Rha, Glc, and GlcA in the proportions of 2.1:1.0:0.3:0.5:0.4:0.9. Among them, GP-B1 treatment had a significant inhibitory effect on the growth of melanoma B16 in vivo and in vitro. Meanwhile GP-B1 could increase the relative spleen weight and stimulate the splenocyte proliferation alone or combined with ConA. Moreover, GP-B1 treatment induced an evident increase in the level of serum TNF-α, IFN-γ, and IL-12 and a reduction for IL-10 production. These results indicate that the antitumor effects of GP-B1 are associated with immunostimulation.

1. Introduction

Gynostemma pentaphyllum Makino, also called jiaogulan, is an herbaceous vine of the family Cucurbitaceae (cucumber or gourd family) indigenous to the southern reaches of China, northern Vietnam, southern Korea, and Japan (Hu, Chen, & Xie, 1996; Rujjanawate, Kanjanapothi, & Amornlerdpison, 2004). *G. pentaphyllum* is best known as an herbal medicine reputed to have powerful antioxidant and adaptogenic effects purported to increase longevity. Clinical research has indicated a number of therapeutic qualities of *G. pentaphyllum*, such as lowering cholesterol and high blood pressure, strengthening immunity, and inhibiting cancer growth (Michael & Liu, 2003). For this reason, *G. pentaphyllum* is most often consumed as an herbal tea, and is also available as an alcohol extract and in capsule or pill form. It is claimed that drinking herb tea of *G. pentaphyllum* could promote health and alleviate the severity of many disorders. Its chemical components contain mainly dammarane-type glycosides, which are identical to some of those found in expensive ginseng, and hence, cheap *G. pentaphyllum* has attracted much interest as a substituted medicinal plant for ginseng and be regarded as "second ginseng" (Cui, Eneroth, & Bruhn, 1999).

Recently, polysaccharide obtained from *G. pentaphyllum* has attracted great attention owing to its antitumor activities (Zhou, Liang, & Hu, 2001), anti-gastric ulcer effect (Rujjanawate et al., 2004), immunomodulatory effect (Qian, Wang, & Tang, 1998, 1999), anti-aging (Luo & Wang, 2005), antioxidant properties (Cai, Zhang, & Wang, 2005; Wang & Luo, 2007), anti-exercise fatigue (Fu, 2000), and treating hyperlipidemia (Birgitte, Per, & Zhao, 1995).

To the best of our knowledge, no information is reported about the antitumor activity of acid polysaccharide from *G. pentaphyllum* herb. Therefore, the present work was undertaken to isolate and characterize the acid polysaccharide form *G. pentaphyllum* and to study its antitumor activity both in vitro and in vivo.

2. Materials and methods

2.1. Materials

The whole *G. pentaphyllum* was purchased from Pingli Country Fiveleaf Gynostemma Institute, Shanxi Province, China. Sepharose CL-6B was purchased from Amersham (Sweden). T-series dextran, DEAE–cellulose, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), cytoxan (CTX), emulsion ConA (concanavalin A) and standard sugars were obtained from

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Sigma (St. Louis, MO, USA). RPMI 1640 medium, fetal calf serum (FCS), penicillin and streptomycin (cell culture grade) were purchased from Gibco (Grand Island, NY, USA). All chemicals and reagents used were of analytical reagent grade.

2.2. Preparation of plant polysaccharide

The dried *G. pentaphyllum* (stem and leaf) were mixed with 95% alcohol to remove lipid and then extracted with distilled water at 90 °C for 2 h each time and three times. The entire water extracts were gathered and concentrated to one fifth of the original volume in a rotary evaporator under reduced pressure, and then the supernatant was precipitated by adding 4 volumes of 95% (v/v) ethanol at 4 °C for 24 h, and the polysaccharide pellets were collected by centrifugation (3000 rpm, 10 min). The extracts were then deproteinated by repeated freeze thawing and the method of Sevag (Staub, 1965), and solubilized in deionized water, then lyophilized to get the crude polysaccharides (CGP).

Size-exclusion and anion-exchange chromatography were used for the fractionation of this preparation. The CGP (22.5 g) was dissolved in distilled water, centrifuged, and then the supernatant was injected to a column (3 cm × 30 cm) of DEAE–cellulose, eluting at a flow rate of 1 ml/min successively with distilled water and a gradient of 0 → 1 mol/l NaCl. The fractions obtained were combined according to the total carbohydrate content quantified by the phenol–sulfuric acid method. The main peak was further fractionated on a Sepharose CL-6B column (2.6 cm × 100 cm) eluted with 0.15 M NaCl to yield three completely separated fractions (one neutral polysaccharide: GP-I; two acidic polysaccharides: GP-B1 and GPC-1). Three main fractions were collected, dialyzed and lyophilized to get white purified polysaccharides (GP-I, 2.3 g, 10.22% of CGP; GP-B1, 1.9 g, 8.44% of CGP; GPC-1, 453.2 mg, 2.01% of CGP). Subsequent physicochemical and biological experiments would be focused on two acidic polysaccharide fractions.

2.3. Analysis of purified polysaccharides

Total sugar content was estimated by the phenol-sulfuric acid assay using D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein contents of the purified polysaccharides were measured according to Bradford's method, using bovine serum albumin (BSA) as the standard (Bradford, 1976). Total uronic acid content was determined by photometry with m-hydroxybiphenyl at 523 nm (Blumenkrantz & Asboe-Hansen, 1973), using GalA as standard.

Molecular weight was determined by high performance gel permeation chromatography on a Angilent 1100 liquid chromatography instrument (USA). TSK-G3000 column (7.8 mm ID × 30.0 cm) was maintained at 35 °C and the mobile phase was 0.1 mol/l Na_2SO_4 (flow rate = 0.5 ml/min), and detected by a RID-10A detector. The samples were dissolved in 0.05 mol/l Na_2SO_4 to reach a final concentration of 1 mg/ml and the sample solution was filtered through 0.45 μm filter membrane before injection (20 μl). The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (T-2000, T-70, T-40, T-20, and T-10). Calculation of the molecular weights of samples was carried out using the Angilent GPC software (USA).

The polysaccharide (3 mg) was hydrolyzed with 2 M TFA at 110 °C for 8 h and conversion of hydrolysate into alditol-acetates as previously described method (Honda, Suzuki, Kakehi, Honda, & Takai, 1981), the resulting alditol-acetates were analyzed by GC using a Vavian 3400 instrument (Hewlett-Packard, Component, USA), and detected with a flame ionization detector (260 °C), the column temperature was increased from 170 to 215 °C in a rate of 2 °C/min then hold on 5 min.

2.4. Cell lines and culture conditions

Human chronic myeloid leukemia K-562, breast adenocarcinoma MCF-7, colon adenocarcinoma HT-29, hepatocellular carcinoma HepG2 and mouse melanoma B16 cell lines were purchased from Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI 1640 with 10% FCS, 100 U/ml penicillin and 100 mg/ml streptomycin in 25-cm^2 culture flasks at 37 °C in humidified atmosphere with 5% CO_2. Cells at the exponential growth phase were harvested from the culture flasks by trypsination and centrifuging at 180 × *g* for 3 min, and then resuspended in fresh medium at 0.5 – 2 × 10^5 cells/ml (depending on the cell line).

2.5. Cell proliferation assay

The effect of drug on the proliferation of cancer cells was determined with the colorimetric MTT assay (Mosmann, 1983). Briefly, the cell suspension at an initial density of 0.5–2 × 10^5 cells/ml was dispensed into 96-well plates and allowed to incubate with 5% CO_2 at 37 °C for 24 h. They were subsequently exposed to drug at concentrations ranging from 25 to 400 μg/ml in a total 200 μl of RPMI 1640 medium containing 10% FCS. Negative controls were treated with the medium only. 20 μl of MTT was added 48 h later to a final concentration of 0.5 mg/ml. After incubation at 37 °C for 4 h, the supernatant was aspirated and 150 μl of DMSO was added to each well to dissolve the formed blue formazan crystals. The absorbance was measured at a wavelength of 545 nm with a reference wavelength of 650 nm by a 96-well microplate reader (Molecular Devices Co., USA.). The proliferation rate was calculated according to the following formula: (A545 of experimental group/A545 of negative group × 100%). The mean inhibitory concentration (IC_{50}) was calculated from the dose–response plots obtained from three independent experiments, each with three replicates. Statistical significance was determined using a Student's *t*-test. Variance of *P*-values obtained was calculated by means of a single-factor ANOVA test.

The acidic polysaccharides were also tested on normal mouse bone marrow cells which were isolated from male KM mice and cultured in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal calf serum according to Meng, Hu, Liu, and Sun (2001).

2.6. Animals and in vivo experiment

Mice (C57BL/6), weighing 25 ± 5 g, were maintained in a sterile and ventilated room at a controlled environmental condition (22 ± 2 °C, 60 ± 5% humidity) with 12 h light/dark cycle. Animals were provided with standard pallet diet and had access to tap water ad libitum. The animal experiments were conducted in accordance with the institutional guidelines and the recommendations for the care and use of laboratory animals put forward by the Ethical Committee of the Xi'an Jiao Tong University.

After 2 weeks of acclimatization, male rats were injected with 2 × 10^6 mouse melanoma B16 cells (from the cell culture described above) subcutaneously in footpad to induce tumors. The normal mice received with only 0.9% normal saline (NS) served as normal control and the tumor bearing mice were randomly divided into four groups (10 mice in each group) as bellow: tumor bearing mice orally administrated with GP-B1 at low-dose of 50 mg/kg; tumor bearing mice orally administrated with GP-B1 at high-dose of 100 mg/kg; tumor bearing mice intraperitoneal (i.p.) injected with cytoxan at a dose of 20 mg/kg (positive control); tumor bearing mice orally administrated with 0.9% NS (negative control).

Table 1
Monosaccharide composition, molecular weight, and the content of total sugar, uronic acid and protein in acidic polysaccharides.

Sample	MW (kDa)	Total sugar (%, w/w)	Uronic acid (%, w/w)	Protein (%, w/w)	Sugar components (mol.%)[a]							
					Gal	Ara	Man	Rra	Xyl	Glc	GalA	GlcA
GP-B1	79	95.3	10.7	0.3	3.5	3.2	0.6	0.9	0.3	0.5	0.6	0.4
GP-C1	126	89.5	18.2	7.2	2.1	1.0	0.3	0.5	–	0.4	–	0.9

Gal: galactose; Ara: arabinose; Man: mannose; Rha: rhamnose; Xyl: xylose; Glc: glucose; GaLA: galacturonic acid; GlcA: glucuronic acid.
[a] Quantities of the carboxy-methylated uronic acid are given in mol.%.

All sample was administrated once everyday from the first day. Meanwhile mice in all groups were observed daily for survival and threatening toxicity by test samples and reference drug.

Tumor volume was determined by measuring blindly footpad diameter with calipers on Days 0, 9, 15, 21 and 27, since the beginning of treatment. Tumor volume (mm^3) was estimated by 1/2 ($A \times B^2$), where A and B represent the long and short dimensions, respectively.

Tumor growth delay was determined according to the method of Corbett et al. (1997) and was calculated by the following equation: Tumor growth delay = $T - C$, where T represents median time (in days) required for the treatment group tumors to reach a volume of 100 mm^3 and C represents median time (in days) required for the control group tumors to reach the same size.

On the 27th day, all mice were sacrificed and tumors and spleens were cut and weighed. Spleen index is the ratio of spleen weight (mg) to the body weight of the mouse (g). Blood was collected by heart puncture, centrifuged at 2000 × g for 10 min and the serum was collected for the detection of various cytokines.

2.7. Lymphocyte proliferation assay

Single cell suspension of splenocytes from control and tumor-bearing mice under GP-B1 treatment was prepared as previously described (Xu et al., 2009). Cell proliferation was estimated based on the method of MTT. Briefly, a total of 1×10^5 cells in 200 μl RPMI 1640 medium containing 10% FCS were seeded into each well of a 96-well flat bottom microtiter plate and stimulated with 2.5 μg/ml ConA or medium (control). After the plates were then placed in a 5% CO_2 incubator at 37 °C for 48 h, 50.0 μl of 0.4% MTT was added into each well. The plate was incubated for another 4 h and centrifuged (1400 × g, 5 min) to remove the untransformed MTT carefully by pipetting. Then a total of 200 μl DMSO was added to fully dissolve the colored material. The optical density at 570 nm was measured using a microtiter plate reader. The experiment was performed in triplicate.

2.8. TNF-α, IFN-γ, IL-10 and IL-12 production

The serum collected from different group was detected for TNF-α, IFN-γ, IL-10 and IL-12 level using commercial ELISA kit according to the instructions of kits. The absorbance was measured at 450 nm in an ELISA reader (Bio-Rad, USA). Cytokine quantities in the samples were calculated from standard curves of recombinant cytokines using a regression linear method (Wang et al., 2007).

2.9. Statistical analyses

All experimental data were given as mean ± S.D. Statistical analysis was carried out using the one-way analysis of variances (ANOVA). Post Dunnett test was applied between control, reference drug and test samples using GraphPad Prism 5.0 software, and $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Isolation and characterization of acidic polysaccharide GP-B1

The crude polysaccharide, CGP, was prepared from *G. pentaphyllum* by defatting with 95% EtOH, hot-water extraction, EtOH precipitation and protein removal by Sevag method and dialysis. After CGP was subjected to a DEAE–cellulose chromatography, one netural polysaccharide fraction (CGP-A1) was eluted by 1 column volume (CV) of distilled water, and two acidic polysaccharides (CGP-B1 and CGP-C1) were separated in turn with increasing content of uronic acid by 3 CV of 0 → 1 mol/l NaCl, respectively. Subsequently three fractions were chromatographed on a Sepharose CL-6B column, each giving a single elution peak, named GP-1, GP-B1, and GP-C1, respectively. The main acidic fractions (GP-B1 and GP-C1) were collected for subsequent analyses. The total carbohydrate content, uronic acid and protein of were 95.3%, 10.7%, and 0.3% for GP-B1, and 89.5%, 18.2% and 7.2% for GP-C1. As determined by HPGPC, the GPC profile GP-B1 and GP-C1 all showed a single and symmetrically sharp peak, indicating their homogeneity, with a weight-average molecular weight of 79 and 126 kDa, respectively. The lack of absorbance at 280 nm and its negative reaction in the Bradford's method indicated that GP-B1 contained no protein. In contrast, a week absorption at 280 nm observed in GP-C1 was well in agreement with its quantity analysis for protein by Bradford's method, with a value of 7.2 percentage of total weight. The sugar composition determined by GC showed that CP-B1 was composed mainly of Gal, Ara, Man, Rha, Xyl, Glc, GalA and GlcA in a molar ration of 3.5:3.2:0.6:0.9:0.3:0.5:0.6:0.4 and GP-C1 consisted of Gal, Ara, Man, Rha, Glc, and GlcA in the proportions of 2.1:1.0:0.3:0.5:0.4:0.9 (Table 1).

3.2. In vitro effects of acidic polysaccharides on the proliferation of different cancer cells and its cytotoxicity to normal cells

For the purpose of efficient access to antitumor ingredients, in vitro antiproliferative activity against five tumor cell lines (K-562, MCF-7, HT-29, HepG2 and B16) and normal mouse bone marrow cells was evaluated by MTT method. The data from Table 2 indicated two acidic polysaccharides had certain inhibition effect for various tumor cells, almost with a lower IC_{50} value (<200 μg/ml) except for GP-C1 on K-562. Particularly, GP-B1 had a significant antiproliferative activity against melanoma B16 with the IC50 values of 65.4 μg/ml. In addition, GP-B1 exhibited a significant lower cytotoxicity to normal mouse bone marrow cells, even at the high dose of 400 μg/ml, where GP-B1 caused no more than 30% growth

Table 2
IC_{50} values for acidic polysaccharides on the proliferation of cancer cell lines.

Sample	IC_{50} values (μg/ml)				
	K-562	MCF-7	HT-29	HepG2	B16
GP-B1	133.9	153.2	167.3	187.4	65.4
GP-C1	>200	128.4	156.4	122.4	152.5

Values represent the mean ± S.D. ($n = 3$).

Table 3
Effect of acidic polysaccharide GP-B1 on tumor growth and spleen index in vivo.

Group	Dose (mg/kg)	Tumor weight (g)	Tumor growth delay (day)	Tumor inhibition (%)	Spleen index (mg/g)
Normal control	–	–	–	–	4.52[a]
Negative control	–	3.2	0		3.49
CTX	20	0.5[b]	5[b]	84.4	2.45[a]
Polysaccharide (GP-B1)	50	1.7[b]	2[a]	46.9	4.28[a]
	100	1.2[b]	3[a]	62.5	4.62[a]

Data are presented as mean ± S.D. values based on 10 mice in each group.
[a] Significant differences compared to the negative control were evaluated using Student's *t* test $P < 0.01$.
[b] Significant differences compared to the negative control were evaluated using Student's *t* test $P < 0.001$.

inhibition (Fig. 1). Therefore this level of cytotoxicity of GP-B1 do not have negative side-effects for inhibiting proliferation of normal cells and is much lower than that against the cancer cells. Based on the above results of bioactive guided screening for efficient anti-cancer ingredients, the effect of GP-B1 against the melanoma B16 would be focal point for following investigation in both in vivo and in vitro experiments.

3.3. In vivo anti-tumor effects of acidic polysaccharide GP-B1 on melanoma-B16-bearing mice

Melanoma-B16-bearing mice were used to evaluate the in vivo anti-tumor effects of acidic polysaccharide GP-B1. Due to the fast growth of tumor, the mice in model control group gradually exhibited a series of weak appearance, such as the lost of appetite, the reduced activity and the body weight with dim hairs. Whereas these behavior and conditions were meliorated in the mice treated with GP-B1. As shown in Table 3, the growth of melanoma B16 tumor in the model mice was significantly suppressed by GP-B1 with the inhibition rate of 46.9% and 62.5% at the dose of 50 and 100 mg/kg body weight, respectively. Meanwhile, tumor delay time of CTX and two GP-B1 treated groups had a noticeable prolong than that of model control ($P < 0.01$ or $P < 0.001$.). Supporting this observation, GP-B1 treatment decreased the average size of tumor volume by about 58% and 76% compared with that of negative control (Fig. 2). As expected, the positive control drug CTX obviously inhibited tumor growth and simultaneously decreased the spleen weight compared to the negative control group ($P < 0.01$). It is notable that the spleen index of the mice treated with the GP-B1 are higher than those of the model mice treated with or without CTX, which is close to the normal control group, suggesting that GP-B1 treatment caused no damage to the immunological function in tumor-bearing mice.

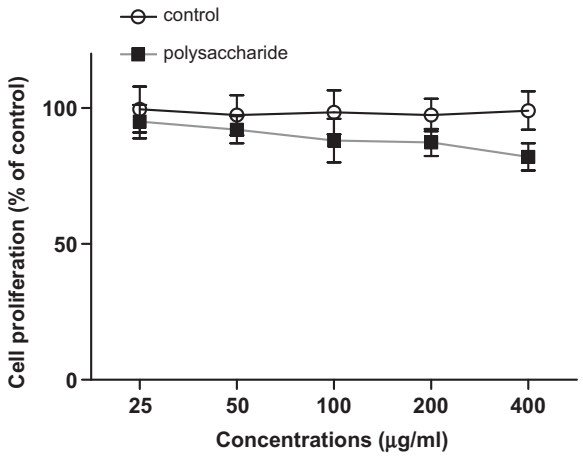

Fig. 1. Inhibitory effect of acidic polysaccharide GP-B1 on the proliferation of normal mouse bone marrow cells. Data are presented as mean ± S.D ($n = 3$).

3.4. In vivo effects of acidic polysaccharide GP-B1 on lymphocyte proliferation of melanoma-B16-bearing mice

Lymphocytes proliferation was studied as an evidence of immunomodulation. Therefore, we evaluated the effects of GSPs on lymphocytes proliferation. As shown in Fig. 3, GP-B1 could cause a significant increase in dose-dependent proliferation of the

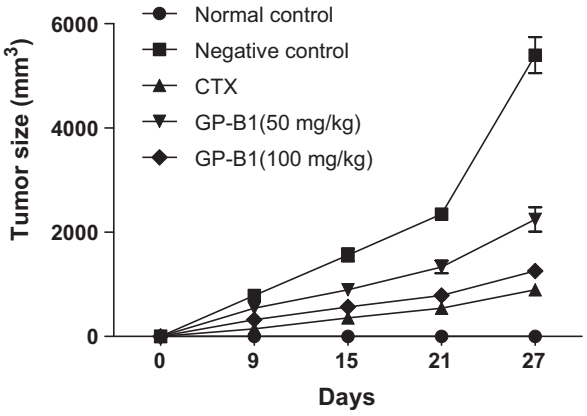

Fig. 2. Inhibitory effect of acidic polysaccharide GP-B1 on B16-induced melanoma tumor growth in C57BL/6 mice. Data are presented as mean ± S.D. values based on 10 mice in each group.

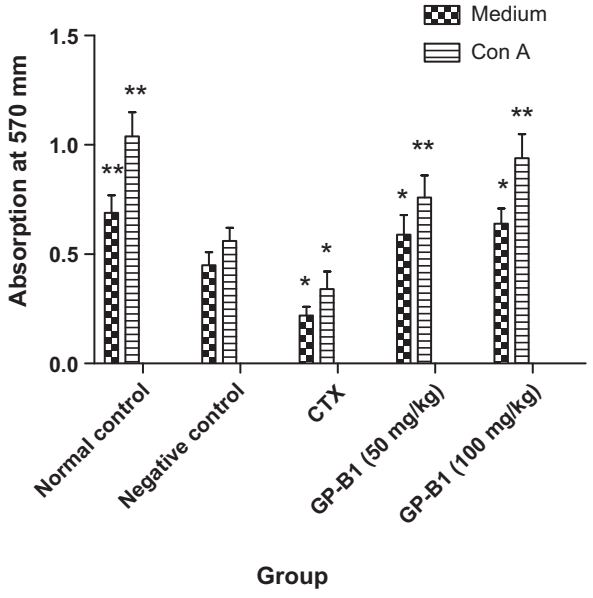

Fig. 3. Effect of acidic polysaccharide GP-B1 on lymphocyte proliferation in melanoma-B16-bearing mice. Data are presented as mean ± S.D. values based on 10 mice in each group. Significant differences compared to the negative control were evaluated using Student's *t* test: $^{*}P < 0.05$ and $^{**}P < 0.01$.

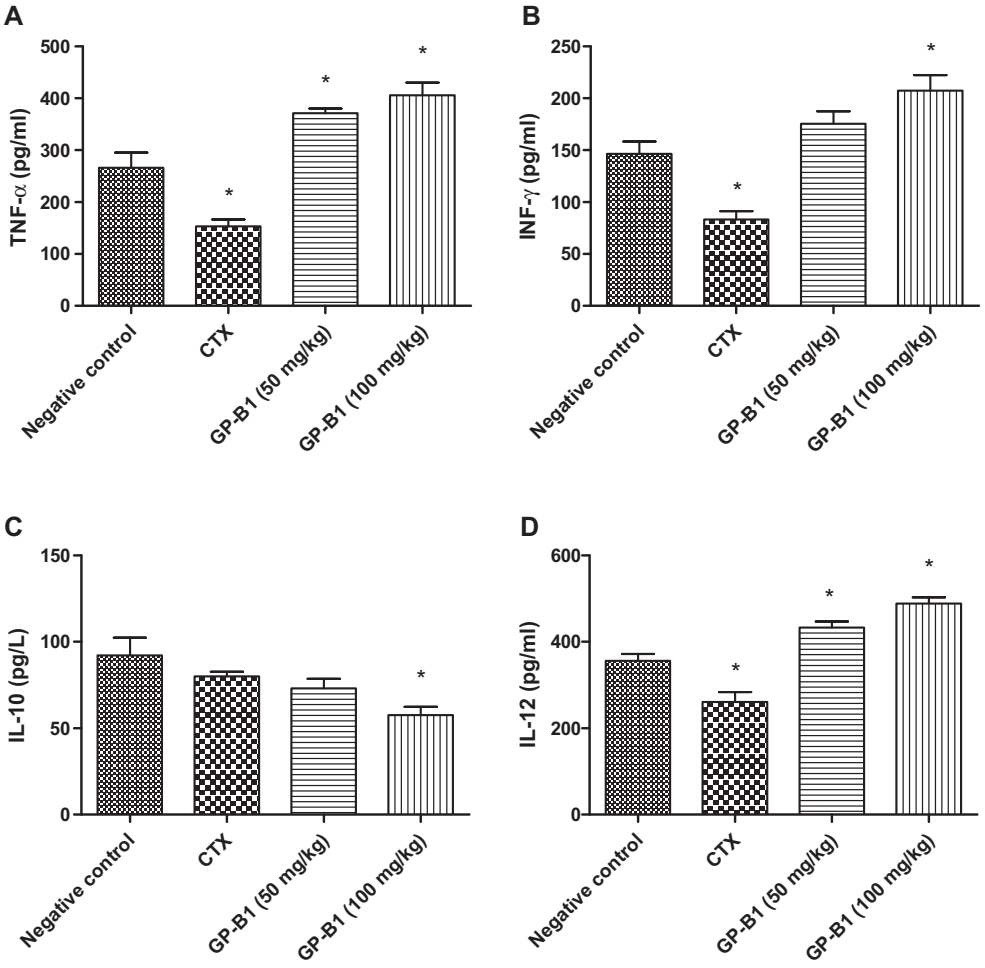

Fig. 4. Effect of acidic polysaccharide GP-B1 on serum TNF-α, IFN-γ, IL-10 and IL-12 production in melanoma-B16-bearing mice. Data are presented as mean ± S.D. values based on 10 mice in each group. Significant differences compared to the negative control were evaluated using Student's *t* test: $^{*}P < 0.05$.

mouse spleen cells in the presence of ConA as mitogens for lymphocytes, as compared with only the ConA-treated group. The results showed that GP-B1 could corporate with ConA to significantly augment ConA-induced lymphocyte proliferation in vitro. Simultaneously GP-B1 itself also exhibited a moderate stimulation effect on lymphocytes proliferation. However, ConA-stimulated splenocyte proliferations in the CTX-treated group were significantly lower than those of the model control ($P < 0.05$).

3.5. *In vivo effects of polysaccharide on serum TNF-α, IFN-γ, IL-10 and IL-12 production of melanoma-B16-bearing mice*

Since cytokines play a prominent role in the development of immune response, we investigated the effect of GP-B1 on serum TNF-α, IFN-γ, IL-10 and IL-12 production in melanoma-B16-bearing mice by MTT assay. As shown in Fig. 4, the levels of TNF-α, IFN-γ, and IL-12 were significantly decreased in CTX-treated mice as compared to those of the model control ($P < 0.05$). However, GP-B1 markedly promoted TNF-α, IFN-γ and IL-12 production in serum of melanoma-B16-bearing mice at two doses ($P < 0.05$). In addition, IL-10, an immunosuppressive cytokine, decreased significantly after administered with GP-B1 compared with that of negative control ($P < 0.05$) at the dose of 100 mg/kg.

4. Conclusion

Previous reports had documented that *G. pentaphyllum* crude extract or water-soluble neutral polysaccharide has anticancer effect. In this investigation, one neutral polysaccharide (GP-I) and two acidic polysaccharides (GP-B1 and GP-C1) had been achieved by ion-exchange and gel-filtration chromatography. MTT assay proved that GP-B1 had a potent anti-proliferation activity on melanoma B16 cells, with the lowest IC_{50} value of 65.4 μg/ml and had no toxicity on the normal mouse bone marrow cells, even at the high dose of 400 μg/ml, indicating its potential application value in the therapy for cancer. Furthermore GP-B1 administrated to melanoma B16-bearing mice could obviously inhibit the tumor growth and improve the immune organ status.

In recent years, increasing the human body's immunity in defending against tumors has been very popular. Particularly the cellular immunity mediated by Th 1 help cells plays an important role in antitumor defense. Some polysaccharides have been shown to promote specific and non-specific immune response in different ways, and their antitumor activity might be achieved by improving immunity (Sullivan, Smith, & Rowan, 2006). Therefore the discovery and identification of new antitumor drugs from traditional Chinese herbs, which can potentiate the immune function, has become an important goal of research in immunopharmacology and oncotherapy (Yuan, Song, Li, Li, & Dai, 2006). In light of

this, we further investigated the effect of GP-B1 on the immunity in tumor-bearing mice to analyze the underlying mechanism of their antitumor activity. The capacity to elicit an effective T-cell immunity can be shown by the stimulation of lymphocyte proliferation response (Marciani et al., 2000). The proliferation assay showed that GP-B1 could significantly promote the ConA-stimulated splenocyte proliferation in tumor-bearing mice, while the positive control CTX with high tumor inhibitory rate had immunosuppressive effect on splenocyte proliferation. The results indicated that GP-B1 could significantly increase the activation potential of T-cells and enhance the cell-mediated immunity in tumor-bearing mice.

Cytokines regulate both cellular and humoral immune responses by affecting immune cell proliferation, differentiation and functions, which is divided two groups, one is proinflammatory factor, and another is antiflammatory factor. TNF-α, IFN-γ, and IL-12 are the major important proinflammatory and immunoregulatory molecules secreted by Th1 help cell with antitumor and immunomodulatory properties. In contrast, IL-10 is an immunosuppressive cytokine, which could diminish several Th1 cell-mediated responses (Rennick, Berg, & Holland, 1992). Increased level of IL-10 is involved in tumor-induced immunosuppression by inhibiting lymphocyte proliferation. In the present study, serum TNF-α, IFN-γ, and IL-12 level in tumor-bearing mice was significantly increased by GP-B1, whereas the serum IL-10 production was reduced. The increase of proinflammatory factor and the reduction of antiflammatory factor may also explain the antitumor properties of GP-B1.

In conclusion, GP-B1, an acidic polysaccharide, from *G. pentaphyllum* could not only significantly inhibit the growth of tumor in vitro and in vivo, but also remarkably increase splenocytes proliferation and the level of serum TNF-α, IFN-γ, IL-10 and IL-12 in tumor-bearing mice, which indicated that the GP-B1 could improve cellular immune response. The above results suggested that the antitumor activity of this acidic polysaccharide might be achieved by improving immune response, and GP-B1 could act as antitumor agent with immunomodulatory activity.

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