

Anti-tumour effects of polysaccharide extracted from *Acanthopanax senticosus* and cell-mediated immunity

QINGLONG MENG¹, JINGZHI PAN², YAJING LIU³, LI CHEN⁴ and YUEYING REN¹

¹College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun, Jilin 130118;

²Tuberculous Meningitis Research Center, Infectious Disease Hospital, Changchun, Jilin 130123;

³College of Plant Science, Jilin University, Changchun, Jilin 130062; ⁴Innovation and Development Centre of Small and Medium Enterprises, Siping, Jilin 136000, P.R. China

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Abstract. *Acanthopanax senticosus*, also known as Siberian ginseng, is widely distributed throughout northern Asia and used in traditional Chinese medicine; it has been reported to prevent a number of diseases. However, the association between the antitumour and immunostimulatory activities of polysaccharide extracted from *A. senticosus* (ASPS) remains to be elucidated. The aim of the present study was to investigate the anti-tumour and immunomodulatory effects of polysaccharide extracted from ASPS on Crocker sarcoma S₁₈₀, hepatic carcinoma H₂₂ and uterine cervical carcinoma U₁₄ tumour cell lines implanted in mice. High performance liquid chromatography, gas chromatography and infrared spectroscopy were used to analyse the monosaccharide composition of ASPS. The monosaccharide composition of ASPS (Arabic candy: Xylose: Glucose: Mannose) was 7.1:22.3:7.6:1.0. On day 0, female Kunming mice, were injected subcutaneously with 1×10⁸ tumour cells in 0.2 ml. The inoculated mice were subsequently divided into five groups (10 mice/group) as follows: Model group, treated with normal saline; positive control group, treated with 30 mg/kg cyclophosphamide (CTX); and three treatment groups, treated with 200, 100 or 50 mg/kg ASPS. Non-inoculated mice were divided into the normal group, which was treated with normal saline, and the negative control group, which was treated with 200 mg/kg ASPS (n=10/group). CTX and ASPS were administered intragastrically once daily for 10 days. All mice were sacrificed on day 11. ASPS was observed to have an inhibitory effect on the growth of S₁₈₀, H₂₂ and U₁₄ cells in solid and ascites tumour-bearing mice. Serum interleukin (IL)-2 and IL-12 levels were significantly increased in S₁₈₀ solid tumour-bearing mice treated with

200 or 100 mg/kg ASPS compared with mice in the normal, control and model groups (P<0.05), whereas serum IL-2 and IL-12 levels were significantly decreased in the cyclophosphamide treatment group compared with the normal, control and model groups (P<0.05). No significant difference in serum levels of tumour necrosis factor-α level was observed between any groups. In S₁₈₀ and U₁₄ solid tumour-bearing mice, no significant differences in serum levels of interferon (INF)-γ level in were observed between groups; however, in H₂₂ solid tumour-bearing mice, treatment with ASPS significantly increased serum INF-γ compared with the positive control group (P<0.05). The results may provide a basis for the potential application of ASPS in clinical treatment for cancer.

Introduction

Malignant cancer is a serious disease that has a negative impact on human health worldwide (1-3). Clinical treatment typically comprises chemically synthesized medicines; however, these are very expensive and often have serious side effects (4-6). Recently, several studies have reported that some agents that are currently used to promote immunity may also inhibit tumour growth (7-9). Research into the activity of natural products and their potential for as cancer treatments is therefore of great medical importance (10,11).

Acanthopanax senticosus, also known as Siberian ginseng, is a perennial xylophyta species in the family of Araliaceae, prefers warm and wet habitats. It is widely distributed in the mountainous broad-leaved forests, mixed forests and forest edges of eastern Hokkaido, Korean Peninsula, northern China and Siberia. *A. senticosus* has been used in traditional Chinese medicine as an adaptogen due to its pharmacological effects, including anti-bacterial, anti-cancer, anti-inflammatory, anti-gout, anti-hepatitis, anti-hyperglycemic, anti-leishmanicidal, anti-oxidant, anti-pyretic effects; other effects include choleric, hemostatic, immunostimulatory, hypocholesterolemic and radioprotectant effects (12-15). It has been shown to protect against oxidative damage and exhibits anti-diabetic activity (16,17). In China, *A. senticosus* is used as a nutritional supplement and a sedative (18). A number of studies have demonstrated that *A. senticosus* has significant therapeutic effects on severe neurosis, fatigue, cardiovascular

Correspondence to: Professor Yueying Ren, College of Chinese Medicinal Materials, Jilin Agricultural University, 2888 Xincheng Street, Changchun, Jilin 130118, P.R. China
E-mail: 24474524@qq.com

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disease (19,20). *A. senticosus* can also improve the immune function (21). Various compounds from *A. senticosus*, including acanthosides, eleutherosides, senticoside, triterpenic saponin, flavon, vitamins, minerals and polysaccharides, have been reported to have diverse biological activities (22,23).

ASPS, as an extract, has been shown to have potent immunomodulatory activity, it is typically concentrated (24,25). ASPS has been demonstrated to improve lymphocyte proliferation (26), induce cytokine actions (27), enhance Toll-like receptor-mediated activation of B cells and improve macrophages phagocytosis (28). However, the association between the antitumour and immunostimulatory activities of ASPS remains to be elucidated. The aim of the present study was to investigate the antitumour effect of ASPS on S_{180} , H_{22} and U_{14} solid and ascites tumour-bearing mice. The immunomodulatory effect of ASPS was also analysed to obtain additional information regarding its underlying mechanisms. ASPS, its composition, proportion of monosaccharides and anti-tumour activity are reported here for the first time.

Materials and methods

Materials. A specimen of *A. senticosus* was foraged by Dr Qinglong Meng from the Liaodong planting base of Chinese Medicinal Materials (Qingyuan, China) and Professor Yueying Ren identified the specimen. The specimen was stored in the Cultivation and Breeding of Medicinal Plants Laboratory in State Administration of Traditional Chinese Medicine (Changchun, China) for experimental applications. The specimen was crushed to powder then passed through mesh sieves, the pass rate of the 20 mesh pharmacopoeia sieve was $\geq 90\%$ and the 80 mesh pharmacopoeia sieve was $\leq 10\%$. The pharmacopoeia sieves were provided by Xinxiang Zhuohang Precision Mesh Filter Co., Ltd. (Henan, China). The specimen then underwent 60°C thermostatic drying for 6 h by blowing air in a thermostatic oven (model, DHG-92438-2; Shanghai Fuma Experimental Equipment Co., Ltd., Shanghai, China). The dried powder was extracted with supercritical CO_2 and placed in distilled water with 0.01–0.05% of mixed enzymes (protease: Cellulose: pectinase in the ratio 3:1.5:0.5). Continuous upstream extraction was performed using distilled water (material: Liquid, 1:18; extraction temperature, 75°C ; extraction time, 2 h). The extracted liquid was filtered, adsorbed with non-polar macroporous resin, washed with 11.2 ml distilled water and filtered through a $0.22\text{ }\mu\text{m}$ membrane. The solid content was concentrated to 20% by 45°C thermostatic drying for 4 h in a thermostatic oven. The ASPS extraction yield was 10.76%.

Compositional analysis. Sephadex-G75 gel filtration (column size, $16\times 500\text{ mm}$; internal diameter, 15 mm) was performed using a high performance liquid chromatography (HPLC) system (ÄKTAFPLC with Fraction Collector Frac-920; all GE Healthcare, Chicago, IL, USA) to purify active molecules from the ASPS extract. The molecules were separated using an Ultrahydrogel™ 500 column ($7.8\times 300\text{ mm}$), which was provided by Waters GmbH (Eschborn, Germany), on the HPLC system using a 0.9% NaCl water solution and distilled water. The column was operated at 35°C . The samples were of 2 mg ASPS were diluted in 1 ml 0.9% NaCl water solution

and a total of $20\text{ }\mu\text{l}$ was injected onto the column. The column was operated at a maximum of 1.6 MPa with a flow rate of 0.5 ml/min. Gas chromatography was then used to analyse the composition of ASPS. Firstly, the samples were hydrated with ethanol (Nanjing Chemical Reagent Co., Ltd., Nanjing, China) for 12 h at 22°C and silylanised with silylate (Shandong Baiqian Chemical Co., Ltd., Shangdong, China) for 30 min at 22°C . Secondly, the samples were analysed with a Varian 7890 gas chromatography spectrometer. The gasification temperature was 300°C . The samples were then separated in high purity nitrogen in a SE-54 column ($15\text{ m}\times 0.2\text{ mm}\times 0.25\text{ }\mu\text{m}$) at 120°C for 2 min, increasing by $8^{\circ}\text{C}/\text{min}$ until the column reached 250°C , then 250°C for 30 min. An infrared spectrometer (Perkin Elmer, Inc., Waltham, MA, USA) was used to record the infrared spectrum of ASPS.

Cell lines. The tumour cell lines Crocker sarcoma S_{180} , hepatic carcinoma H_{22} and uterine cervical carcinoma U_{14} were provided by a drug clinical trial from Jilin Province Cancer Hospital (Changchun, China) and cultured for 7 days at 37°C in the Cultivation and Breeding of Medicinal Plants Laboratory.

Mice. A total of 360 female Kunming mice, 6–7 weeks old and weighing 18–22 g, were provided by the Changchun Institute of Biological Products (Changchun, China; license number: SCXKJi 2013-0001). The mice were maintained in clean plastic cages in the laboratory of the College of Chinese Medicinal Materials at Jilin Agricultural University (Changchun, China). The temperature was controlled at $22\pm 2^{\circ}\text{C}$ with a 12 h light/dark cycle and relative humidity of 50–60%. Standard rodent chow and water were freely accessible. All mice were maintained for an acclimatization period of ~ 7 days under normal laboratory conditions. All mice were treated according to the National Regulations on the Usage and Welfare of Animals (29) and study protocols were approved by the Animal Ethical and Welfare Committee of the College of Chinese Medicinal Materials, Jilin Agricultural University prior to the experiments (approval no. 2013-006).

Inhibition rate, immune organ index and cytokine levels of solid tumour-bearing mice. On day 0, each mouse was injected subcutaneously with 1×10^8 tumour cells in 0.2 ml normal saline. A total of 50 Kunming mice used for each cell line and the same number of tumour cells were injected regardless of the cell line used. The inoculated mice were subsequently randomly divided into five groups (10 mice/group) as follows: Model group, treated with normal saline; positive control group, treated with cyclophosphamide (CTX; 30 mg/kg); and treatment groups, administered with 200, 100 or 50 mg/kg ASPS. Non-inoculated mice were divided into the normal group, treated with normal saline, and the negative control group, treated with 200 mg/kg ASPS ($n=10/\text{group}$). CTX and ASPS were administered intragastrically once daily for 10 days. All mice were sacrificed on day 11. Mice were weighed every 2 days throughout the treatment period and, following sacrifice, the tumours, spleens and thymuses were harvested and weighed. The tumour growth inhibition rates and immune organ indices were calculated using the following equations: Inhibition rate (%) = [(Mean tumour weight in the model group – mean tumour weight in the treatment group) / mean

Table I. Inhibitory effect of ASPS in S₁₈₀ solid tumour-bearing mice.

Group	Dose (mg/kg) and agent	Weight (g)		Tumour weight (g)	Inhibition rate (%)
		Pre-treatment	Post-treatment		
Model	0	22.09±1.34	30.45±2.24	2.69±0.45	
Positive control	30 CTX	22.17±1.28	27.31±2.16 ^a	0.78±0.29 ^a	71.00
High dose	200 ASPS	21.98±1.19	32.73±1.93 ^b	1.63±0.32 ^{a,b}	39.41
Medium dose	100 ASPS	22.04±1.17	31.06±1.37 ^b	1.49±0.45 ^{a,b}	44.61
Low dose	50 ASPS	21.97±1.25	32.69±2.04 ^b	1.59±0.41 ^{a,b}	40.89

^aP<0.01 vs. model group; ^bP<0.01 vs. positive control group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide.

Table II. Inhibitory effect of ASPS in H₂₂ solid tumour-bearing mice.

Group	Dose (mg/kg) and agent	Weight (g)		Tumour weight (g)	Inhibition rate (%)
		Pre-treatment	Post-treatment		
Model	0	21.99±1.56	29.03±1.98	3.08±0.74	
Positive	30 CTX	21.95±1.38	26.73±2.07 ^a	0.99±0.36 ^a	67.86
High dose	200 ASPS	22.01±1.62	30.95±2.33 ^b	1.71±0.41 ^{a,b}	44.48
Medium dose	100 ASPS	21.97±1.48	31.29±2.67 ^b	1.75±0.51 ^{a,b}	43.19
Low dose	50 ASPS	22.02±1.51	30.82±2.18 ^b	1.87±0.65 ^{a,b}	39.29

^aP<0.01 vs. model group; ^bP<0.01 vs. positive control group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide.

tumour weight in the model group] x100. Immune organ index = Organ weight (mg) / body weight (g) (30,31). Blood was harvested from mice in each group and centrifuged at 1,409 x g at room temperature for 10-15 min. The serum levels of tumour necrosis factor (TNF)- α (cat. no. MTA00B), interleukin (IL)-2 (cat. no. M2000), IL-12 (cat. no. M1270) and interferon (INF)- γ (cat. no. MIF00) were determined using commercial ELISA kits according to the manufacturer's protocols (R&D Systems, Inc., Minneapolis, MN, USA).

Increased life span (ILS) of ascites tumour-bearing mice. A total of 50 female Kunming mice were inoculated, divided into groups and treated as above. For ascites tumour-bearing mice, there were no non-inoculated mice ILS was calculated using the following equation: ILS %=[(Mean survival days of treated group-mean survival days of model group)/mean survival days of model group] x100 (31).

Test of acute toxicity. A total of 40 Kunming mice (20 male and 20 female; 6-7 weeks old; 20-22 g) were randomly divided into two groups: Administration and blank (n=20 in each). All mice were fasted for 12 h prior to experimentation, but were not deprived of water. Mice in the administration group were treated with 10 g/kg ASPS once daily for 14 days. Mice in the blank group were administered with 0.2 ml normal saline daily. Observations of the limb flexibility, feeding, diarrhoea, death, hair finish (whether the hair is dry and falling out), and stool and urine secretions were recorded. All mice were sacrificed on day 15. The major organs, including the heart, liver,

spleen, lungs and kidneys of the mice, were tested for toxicity following the sacrifice of the mice.

Statistical analysis. Data are presented as the mean \pm standard deviation. Differences between groups were analysed using the Least-Significant Difference post-test. Statistical analyses were conducted using SPSS (version 17.0; SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

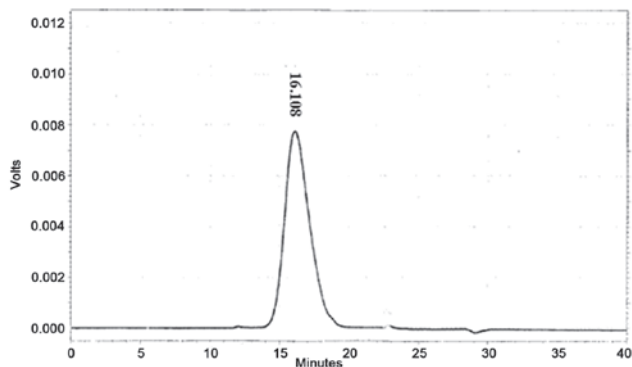
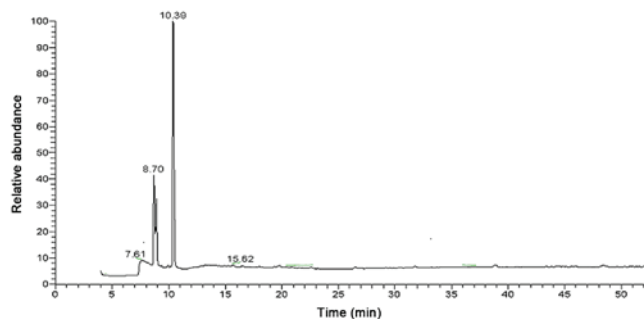
Composition of ASPS. The single, narrow, symmetrical peak of the analysed ASPS sample revealed its homogeneous composition (Fig. 1). The molecular weight of ASPS was 10 kDa and its monosaccharide composition was demonstrated to be (Arabinose: Xylose: Glucose: Mannose)=7.1:22.3:7.6:1.0 (Fig. 2).

Tumour growth inhibitory effect of ASPS in solid tumour-bearing mice. Treatment with high, medium and low dose ASPS had a significant inhibitory effect on tumour growth in mice inoculated with S₁₈₀ (Table I), H₂₂ (Table II) and U₁₄ (Table III) compared with the model group (all P<0.01). The greatest inhibitory effect in S₁₈₀ and U₁₄ tumour-bearing mice was achieved with the medium dose (100 mg/kg; Tables I and III), whereas the high dose (200 mg/kg) had the greatest effect in H₂₂ tumour-bearing mice (Table II).

Table III. Inhibitory effect of ASPS in U₁₄ solid tumour-bearing mice.

Group	Dose (mg/kg) and agent	Weight (g)		Tumour weight (g)	Inhibition rate (%)
		Pre-treatment	Post-treatment		
Model	0	21.82±1.76	29.65±2.05	2.89±0.64	
Positive control	30 CTX	21.98±1.91	26.47±2.19 ^a	0.93±0.41 ^a	67.82
High dose	200 ASPS	22.08±1.62	29.87±2.40 ^b	1.65±0.46 ^{a,b}	42.91
Medium dose	100 ASPS	22.03±1.81	30.53±2.81 ^b	1.61±0.58 ^{a,b}	44.29
Low dose	50 ASPS	21.94±1.71	30.12±2.17	1.82±0.53 ^{a,b}	37.02

^aP<0.01 vs. model group; ^bP<0.01 vs. positive control group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide.

Figure 1. Chromatogram of *Acanthopanax senticosus* polysaccharide.Figure 2. Gas chromatogram of the monosaccharide composition of *Acanthopanax senticosus* polysaccharide.

Effect of ASPS on immune organ index in solid tumour-bearing mice. Treatment with high, medium and low doses of ASPS increased the immune organ indices in S₁₈₀, H₂₂ and U₁₄ solid tumour-bearing mice. Spleen and thymus indices in the CTX group were significantly decreased compared with the normal, control and model groups for all cell lines (P<0.01; Figs. 3-5). Spleen and thymus indices in the high, medium and low ASPS treatment groups were significantly increased compared with the positive control group for all cell lines (P<0.01; Figs. 3-5).

Effect of ASPS on cytokines levels in solid tumour-bearing mice. Treatment with high, medium and low doses of ASPS significantly increased serum IL-2 levels in S₁₈₀, H₂₂ and U₁₄ solid tumour-bearing mice compared with the normal, control,

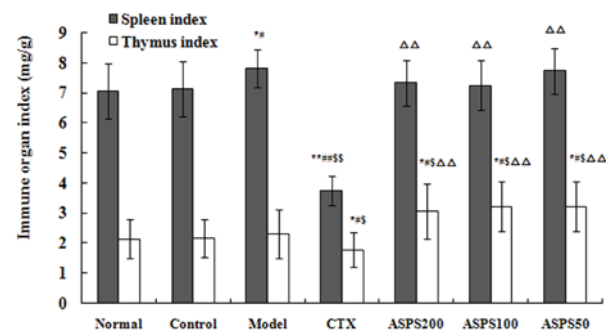


Figure 3. Immune organ indices of ASPS in S₁₈₀ solid tumour-bearing mice. *P<0.05 and **P<0.01 vs. normal group; #P<0.05 and ##P<0.01 vs. control group; [§]P<0.05 and ^{§§}P<0.01 vs. model group; ^{ΔΔ}P<0.01 vs. CTX group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.

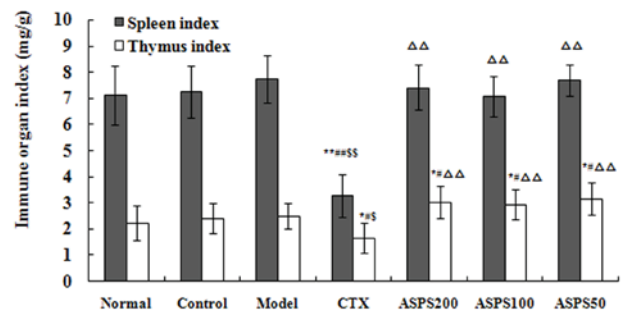


Figure 4. Immune organ indices of ASPS in H₂₂ solid tumour-bearing mice. *P<0.05 and **P<0.01 vs. normal group; #P<0.05 and ##P<0.01 vs. control group; [§]P<0.05 and ^{§§}P<0.01 vs. model group; ^{ΔΔ}P<0.01 vs. CTX group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.

CTX and model groups (S₁₈₀, P<0.05, P<0.05 and P<0.01, respectively; H₂₂ and U₁₄, all P<0.01; Fig. 6). IL-2 levels were significantly decreased in the CTX group compared with the normal, control and model groups (S₁₈₀, P<0.01, P<0.05 and P<0.05, respectively; H₂₂ and U₁₄, all P<0.01; Fig. 6). High and medium doses of ASPS significantly increased serum IL-12

Table IV. Antitumour effect of ASPS in S_{180} ascites tumour-bearing mice.

Group	Dose (mg/kg) and agent	Survival time (days)	ILS (%)
Model	0	13.67±2.65	
Positive control	30 CTX	18.78±2.68 ^b	37.38
High dose	200 ASPS	15.44±2.70 ^c	12.95
Medium dose	100 ASPS	17.22±2.58 ^b	25.97
Low dose	50 ASPS	16.33±2.12 ^{a,c}	19.46

^aP<0.05 and ^bP<0.01 vs. model group; ^cP<0.01 vs. positive control group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide.

Table V. Antitumour effect of ASPS in H_{22} ascites tumour-bearing mice.

Group	Dose (mg/kg) and agent	Survival time (days)	Increased life span (%)
Model	0	11.67±2.65	
Positive control	30 CTX	16.89±2.09 ^b	44.73
High dose	200 ASPS	15.21±2.39 ^b	30.25
Medium dose	100 ASPS	14.67±2.55 ^b	25.71
Low dose	50 ASPS	13.89±2.57 ^a	19.02

^aP<0.05 and ^bP<0.01 vs. model group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide.

Table VI. Antitumour effect of ASPS in U_{14} ascites tumour-bearing mice.

Group	Dose (mg/kg) and agent	Survival time (days)	Increased life span (%)
Model	0	12.22±3.15	
Positive control	30 CTX	16.89±3.33 ^b	38.22
High dose	200 ASPS	15.44±2.07 ^b	26.35
Medium dose	100 ASPS	14.67±2.92 ^b	20.05
Low dose	50 ASPS	14.11±2.62 ^a	15.47

^aP<0.05 and ^bP<0.01 vs. model group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide.

levels in H_{22} and U_{14} solid tumour-bearing mice compared with the normal, control, CTX and model groups (P<0.01; Fig. 7); furthermore, IL-12 levels were significantly increased with high or medium-dose ASPS treatment in S_{180} tumour-bearing mice compared with the control, CTX and normal groups (P<0.01; Fig. 7). No significant differences in serum TNF- α levels were observed between groups, irrespective of treatment (Fig. 8). No significant differences in serum INF- γ levels were observed in S_{180} and U_{14} solid tumour-bearing mice in

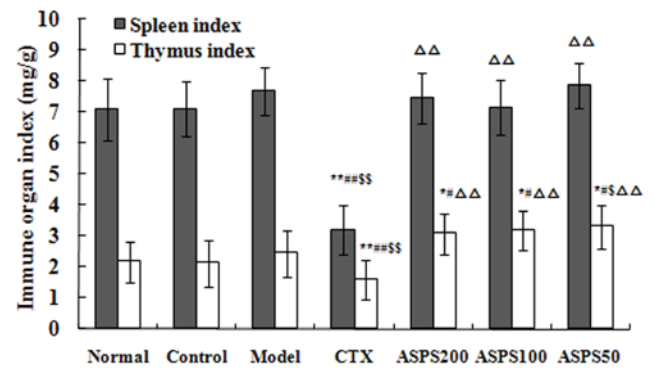


Figure 5. Immune organ indices of ASPS in U_{14} solid tumour-bearing mice. *P<0.05 and **P<0.01 vs. normal group; #P<0.05 and ##P<0.01 vs. control group; \$P<0.05 and \$\$P<0.01 vs. model group; Δ P<0.01 vs. CTX group. ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.

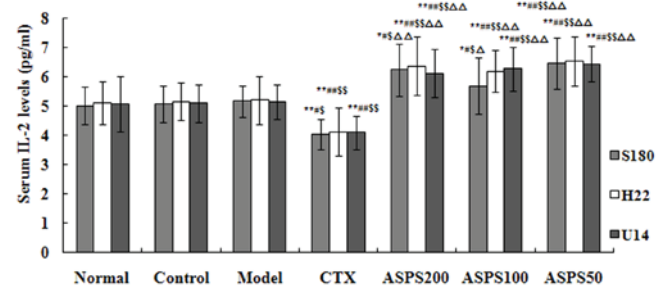


Figure 6. Serum IL-2 levels of ASPS in solid tumour-bearing mice. *P<0.05 and **P<0.01 vs. normal group; #P<0.05 and ##P<0.01 vs. control group; \$P<0.05 and \$\$P<0.01 vs. model group; Δ P<0.01 and $\Delta\Delta$ P<0.01 vs. CTX group. IL, interleukin; ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.

different treatment groups (Fig. 9). However, treatment with high (P<0.05), medium (P<0.01) and low (P<0.05) doses of ASPS significantly increased INF- γ expression in H_{22} solid tumour-bearing mice compared with the positive control group (Fig. 9).

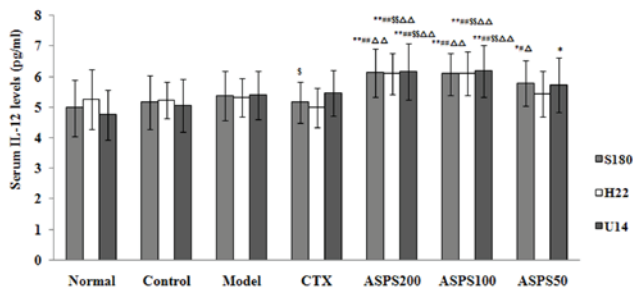
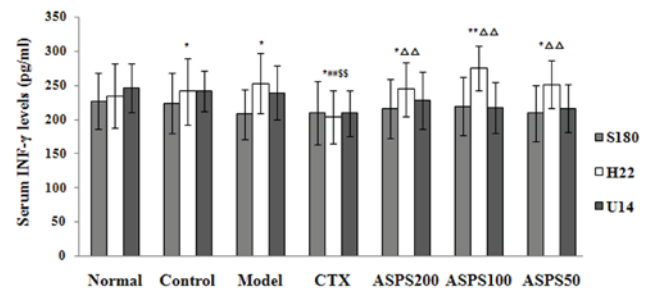
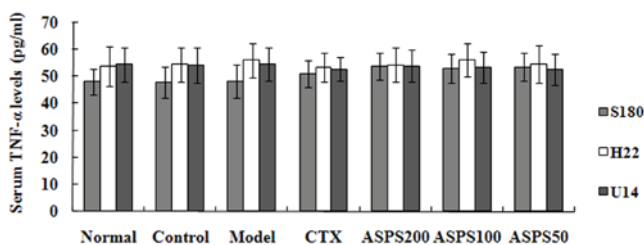
Antitumour effect of ASPS in ascites tumour-bearing mice.

Treatment with medium (P<0.01) and low (P<0.05) doses of ASPS had inhibitory effects on the growth of S_{180} in ascites tumour-bearing mice compared with the model group (Table IV). Treatment with high (P<0.01), medium (P<0.01) and low (H_{22} , P<0.05 and U_{14} , P<0.01) doses of ASPS had an inhibitory effect on the growth of H_{22} and U_{14} in ascites tumour-bearing mice (Tables V and VI). The greatest inhibitory effect in S_{180} tumour-bearing mice was observed with the ASPS medium dose (100 mg/kg; Table IV). The greatest inhibitory effect in H_{22} and U_{14} tumour-bearing mice was observed with the ASPS high dose (200 mg/kg; Tables V and VI).

Test of acute toxicity. All mice in the oral administration groups survived until the end of the 14-day observation period.

Table VII. Body weight changes of mice following oral administration of 10 g/kg *Acanthopanax senticosus* polysaccharide for 14 days.

Group	Sex	Body weight (g)				
		Day 0	Day 2	Day 6	Day 10	Day 14
Administration	Male	19.2±0.94	23.6±1.01	29.1±1.62	32.6±2.04	35.0±2.72
	Female	18.7±0.96	22.1±1.39	26.1±1.45	27.5±2.13	28.9±2.17
Blank	Male	19.4±0.97	23.7±1.01	29.1±1.37	32.6±1.85	33.4±2.33
	Female	18.5±0.82	21.75±1.23	25.5±1.53	27.0±2.27	27.2±2.66

Figure 7. Serum IL-12 levels of ASPS in solid tumour-bearing mice. * $P < 0.05$ and ** $P < 0.01$ vs. normal group; # $P < 0.05$ and ## $P < 0.01$ vs. control group; § $P < 0.05$ and §§ $P < 0.01$ vs. model group; Δ $P < 0.01$ and ΔΔ $P < 0.01$ vs. CTX group. IL, interleukin; ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.Figure 9. Serum INF- γ levels of ASPS in solid tumour-bearing mice. * $P < 0.05$ and ** $P < 0.01$ vs. normal group; ## $P < 0.01$ vs. control group; §§ $P < 0.01$ vs. model group; Δ $P < 0.01$ vs. CTX group. INF, interferon; ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.Figure 8. Serum TNF- α levels of ASPS in solid tumour-bearing mice. TNF, tumour necrosis factor; ASPS, *Acanthopanax senticosus* polysaccharide; CTX, cyclophosphamide; ASPS200, 200 mg/kg ASPS; ASPS100, 100 mg/kg ASPS; ASPS50, 50 mg/kg ASPS.

Mice were sacrificed on day 15 and no visible lesions were observed in the vital organs. These results suggest that mice are able to tolerate >10 g/kg ASPS without adverse effects. According to the standard classification of acute toxicity of chemical substances (32), ASPS is a non-toxic material. No abnormalities in eating, drinking, stool, urine, disposition, mobility (data not shown) or body weight (Table VII) were observed in any groups.

Discussion

Biological response modifiers have previously been used as a tool for inhibiting tumour growth and metastasis (33,34). In the tumour microenvironment, tumour cells often secrete immunosuppressive factors that alter host immune function

and suppress immune response cells (35). The dysregulation of immunity and abnormal immune effector cells may lead to reduced anti-tumour activity (36); therefore, reversing the apoptotic pathway in tumour-induced immune cells has emerged as a method of tumour therapy (37). In the present study, the inhibitory effects of ASPS on tumour growth and its immunomodulatory activity were demonstrated to be independent of dosage. The medium dosage (100 mg/kg) of ASPS had the greatest inhibitory effect on tumour growth in mice. Previous studies have reported that the key determinant of polysaccharide regulatory function is the level in the body rather than the administered dose (38–40). These results suggest that ASPS may regulate immunity levels during normal autoimmune processes.

The immune system is a network comprising cells and organs that protect the body against external attacks. The degeneration and atrophy of immune organs will therefore negatively affect the function of the whole immune system. For example, the spleen filters and serves as a reservoir for blood; if the spleen is damaged or removed, the individual will be more susceptible to infection (41). In the present study, ASPS treatment had a positive effect on thymus and spleen indices in tumour-bearing mice.

Cellular immunity is important for host tumour immunity and cytokines serve an important role in the development of the immune response. IL-2 is a prominent immune factor that is secreted from helper T lymphocytes, natural killer cells, lymphokine activated killer cells and macrophages, all of which are involved in antitumour immunological mechanisms (42).

Inflammatory cytokines, including TNF- α , are secreted by macrophages and serve a role in the activation of T cells and tumour cell recognition (43). IL-12 is secreted by phagocytic antigen-presenting cells, including macrophages and dendritic cells, and is considered to be one of the most essential cytokines in antitumour immunity, serving as a multifunctional cytokine in the early stages of the immune response (44,45). Previous animal studies have demonstrated that IL-12 has potent antitumour and antimetastasis activities, and its effect is most likely mediated via IFN- γ (45,46).

In the present study, ASPS was demonstrated to have an inhibitory effect on the growth of S₁₈₀, H₂₂ and U₁₄ cells in both solid and ascites tumour-bearing mice, potentially via increasing serum IL-2, IL-12 and INF- γ levels. The results may provide a basis for the potential applications of ASPS in clinical treatment for cancer. There were a number of limitations in the present study, including a lack of pharmacodynamic tests for each independent component. During the treatment of tumour-bearing mice, each active component may exert different functions. In future studies, it will be necessary to assess the pharmacodynamic effects of each component of ASPS. In addition, although ASPS is an active component extracted from a natural pharmaceutical, its potential adverse effects require further investigation.

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