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PHYSICOCHEMICAL PROPERTIES AND TUMOR INHIBITION EFFECT OF BITTER GOURD POLYSACCHARIDE

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Abstract

Crude polysaccharide from bitter gourd was purified to obtain the bitter gourd polysaccharide (BGP) fraction. Chemical composition analysis showed that BGP comprised a polysaccharide complex-bound protein peptide with a total polysaccharide content of 92.68% and protein content of 2.33%. Infrared analysis showed that BGP was a typical carbohydrate. BGP significantly inhibited the growth of both, BEL-7402 and HepG-2 human hepatoma cell lines and showed a dose-effect relationship. The overall inhibition effect by BGP was stronger on HepG-2 cells than on BEL-7402 cells. BGP doses of 80, 160 and 320 mg/kg/d significantly inhibited tumor growth in sarcoma S180-bearing mice ($p < 0.01$ or $p < 0.05$), with inhibition rate increasing from 22 to 64%; BGP doses of 160 and 320 mg/kg.d significantly promoted the growth of spleen and thymus in S180-bearing mice ($p < 0.01$), showing a dose-effect relationship.

Introduction

Bitter gourd, the fruit of a vine from the family Cucurbitaceae, is a bitter-tasting vegetable and is part of the daily diet of the Chinese population. Because of its excellent dietary property and significant physiological regulatory function, bitter gourd is also known as a medicinal vegetable (Hu and He 2009), often used in traditional Chinese medicine to prevent or treat variety of diseases such as diabetes mellitus, dysentery, ophthalmodynia, and carbuncles (Hu and He 2009. The physiological regulatory function of bitter gourd is contributed by its active components, such as saponin, polypeptides, and bitter gourd polysaccharides (BGP) (Bradford 1976). So far, the physiological regulatory function of active components from bitter gourd has rarely been investigated. In this study, crude BGP (CBGP) was obtained from bitter gourd by decoction extraction separation method, and the BGP fraction was purified by column chromatography. The physicochemical properties and tumor inhibition activity of the purified BGP fraction were determined for further development and application of BGP.

Materials and Methods

Bitter gourd was cultivated in Shouguang, Shandong province, China. Male Kunming mice (specific-pathogen-free grade, weighing 18 to 22 g) were purchased from the Shanghai experimental animal center, Chinese Academy of Sciences, Shanghai, China. The instruments used included: Agilent 1200 Series high-performance liquid chromatography, evaporative light

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scattering detector (ELSD, Alltech 3300) Alltech, USA; TSKgel G4000PWxL column, Tosoh Bioscience, USA; Thermo Nicolet 6700 Fourier transform infrared (FTIR) spectrometer, Thermo Trace-2000 gas chromatograph (GC), Rtx-5MS capillary column (30 m × 0.32 mm i.d.), Thermo Finnigan, USA; UV-2450 UV-visible spectrophotometer, Shimadzu, Japan; Cell culture incubator, Thermo Scientific, USA; Dextran Standard T-series, Pharmacia; Bradford protein assay kit, Beyotime, Shanghai, China. All reagents used were of analytical grade.

A portion of BGP (2.5 mg) was pelleted with an appropriate amount of potassium bromide and then scanned in the range of 4000 to 400 cm^{-1} using a Nicolet-170X infrared spectrometer.

Whole bitter gourd (1 kg) was mechanically minced with 40 l litre water, boiled, and then filtered. Protein was removed by the Sevag method (Zhou *et al*. 2003), followed by hydrogen peroxide decolorization, ethanol precipitation, and freeze-drying. A pale yellow powder weighing 18.28 g was obtained and designated as CBGP, which was dissolved in water and purified by diethylaminoethyl (DEAE)-cellulose DE52 ion exchange chromatography to obtain purified BGP fraction. After dialysis with water and freeze-drying, a light yellow flocculent powder weighing 7.89 g was obtained, which was designated as BGP.

The polysaccharide content of BGP was determined using the anthrone-sulfuric acid method (Morris 1948). The protein content of BGP was determined by Bradford (1976) assay. The monosaccharide composition of BGP was determined by the aldononitrile acetate derivatization method (Zhou *et al*. 2003).

The molecular weight of BGP was determined by high-performance gel-permeation chromatography (HPGPC)-ELSD analysis. Dextran standard T-series including T-10, T-40, T-70, T-500, and T-2000 (molecular weight: 10000, 40000, 70000, 500000, and 2000000 Da, respectively) were used. A standard curve was prepared using weight-average relative molecular weight *versus* the retention time (tR) of HPGPC-ELSD chromatogram to obtain the linear regression equation (Chen *et al*. 2011).

BEL-7402 and HepG-2 human hepatoma cell lines were separately cultured in RPMI-1640 medium containing penicillin (120 IU/ml), streptomycin (120 U/ml), and 10% fetal bovine serum (inactivated at 60°C for 30 min). The cell cultures were incubated at 37°C in an incubator under an atmosphere of 5% CO_2 with saturation humidity (Zhang *et al*. 2009 and Graves and Cochran 2003).

Tumor cells in the logarithmic growth phase were trypsinized and seeded onto 96-well plates. BGP at final concentrations of 500, 300, 150, or 50 μg/ml (3 wells each) was added to each well of the experimental group, while an equal volume of RPMI 1640 medium was added to each of the 3 negative-control wells. The plates were incubated in an incubator (37°C, 5% CO_2) for 48 hrs, after which, MTT was added. After another 4 hrs of incubation, the absorbance of cell cultures at 570 nm (A570) was measured. Inhibition rate (IR) of cell growth (%) was calculated as [(1 - A570 of the experimental group/A570 of the control group) × 100] (Kim *et al*. 2003, Jacobson *et al*. 2004 and Cheng *et al*. 2003).

Male Kunming mice, weighing 18 to 22 g were subcutaneously injected in the right armpit with S180 cell suspension (5.5×10^7 cells/ml) at a dose of 0.20 ml/20 g. Random grouping (n = 8 each) was conducted 24 h after injection. Mice of the tumor-bearing control group were administered an equal volume of normal saline, while those of the BGP-treatment groups were further divided into high-, intermediate- and low-dose groups for intragastric administration of BGP at 80, 160 and 320 mg/kg/d, respectively. Mice of the positive-control group were intraperitoneally administered 5-fluorouracil (120 mg/kg/d). Each group was administrated the indicated drugs for 10 consecutive days. The mice were euthanized by cervical dislocation on the next day after the completion of drug administration. The animals were dissected, and the tumor, thymus, and spleen were collected and weighed. Tumor inhibition rate and organ indices (for

thymus and spleen) were calculated as follows: tumor inhibition rate (%) = [(Mean tumor weight of the tumor-bearing control group - mean tumor weight of the experimental group)/ mean tumor weight of the tumor-bearing control group] × 100; and organ index (mg/g) = Organ weight (mg)/ body weight (g) (Jiao *et al*. 2011).

Results and Discussion

The purified BGP fraction obtained by the anthrone-sulfuric acid method showed a total polysaccharide content of 92.68% and protein content of 2.33%. Thus, the BGP fraction obtained comprised a polysaccharide complex-bound protein peptide. GC analysis showed that the polysaccharide group of BGP contained 5 monosaccharides (Table 1) including glucose, galactose, rhamnose, arabinose and mannose. HPGPC revealed that the molecular weight of BGP was 1.46×10^5 Da.

Table 1. Monosaccharide composition and molar ratio of BGP (mol%).

Monosaccharide	Rhamnose	Arabinose	Mannose	Glucose	Galactose
BGP	14.8	14.2	20.6	35.2	15.2

BGP, bitter gourd polysaccharide.

FTIR results showed multiple characteristic absorption peaks at 3420, 2928, 2850, 1655, 1420, 1377 and 1081/cm. The peak at 3420/cm was attributed to the stretching vibration of O-H, while those at 2928 and 1420/cm were attributed to the stretching and bending vibrations of C-H, respectively. The large peak at approximately 1655/cm was attributed to water, indicating that BGP could easily absorb water. Characteristic absorption at 1081/cm was attributed to the stretching vibration of C-O-C. Thus, FTIR characteristic absorption analysis of functional groups showed that BGP was a typical carbohydrate.

Cell culture showed that purified BGP significantly inhibited the growth of both, BEL-7402 and HepG-2 human hepatoma cells, showing a dose-effect relationship. At 300 µg/ml BGP dose, IR for BEL-7402 and HepG-2 cells was >30%; as BGP dose reached 500 µg/ml, IR for BEL-7402 and HepG-2 cells exceeded 50%. At higher doses, BGP showed higher IR for HepG-2 than for BEL-7402 cells.

Table 2. Inhibitory effect of bitter gourd polysaccharide (BGP) on BEL-7402 and HepG-2 cells.

Group	Dose (µg/ml)	Inhibition rate (%)	
		BEL-7402	HepG-2
Control	0	0	0
BGP-50	50	6.42 ± 0.11	7.32 ± 0.45
BGP-150	150	10.76 ± 0.46	15.78 ± 0.64
BGP-300	300	31.22 ± 0.91	36.61 ± 0.81
BGP-500	500	55.62 ± 2.88	60.19 ± 3.21

Compared with the tumor-bearing control group, the 80, 160, and 320 mg/kg.d BGP-treated groups showed significant growth inhibition of mouse sarcoma S180 cells ($p < 0.01$ or $p < 0.05$). With an increasing dose, the tumor IR of BGP for mouse sarcoma S180 significantly increased from 22 to 64%, showing a dose-effect relationship. The tumor IR for S180 was lower in the 3 BGP-treated groups than that in the 5-FU-treated control group (Table 3).

Table 3. Inhibitory effect of bitter gourd polysaccharide on mouse sarcoma S180.

Group	Dose (mg/kg/d)	Tumor weight (g)	Tumor inhibition rate (%)
Tumor-bearing control	/	3.04 ± 0.44	/
5-FU control	120	0.88 ± 0.21 **	71.05
BGP	80	2.36 ± 0.53 *	22.37
	160	1.78 ± 0.49 **	41.45
	320	1.08 ± 0.18 **	64.47

Compared with tumor-bearing control: *p < 0.05, ** p < 0.01. 5-FU: 5-fluorouracil.

The results of organ index analysis for S180-bearing mice are shown in Table 4. The spleen index and the thymus index values in the high-dose BGP group were >2.5-fold and 2-fold of that in the tumor-bearing control group, respectively. Compared with the tumor-bearing control group, spleen index and thymus index values in the intermediate- and high dose groups increased to significantly higher levels ($p < 0.01$), showing a dose-effect relationship. This result suggested that BGP has a growth-promoting effect on the spleen and thymus in S180-bearing mice. Presumably, the immune enhancement effect of BGP in mice is relevant to its tumor inhibition effect.

Table 4. Effect of bitter gourd polysaccharide on the spleen and thymus indices in S180-bearing mice.

Group	Dose (mg/kg.d)	Spleen index	Thymus index
Tumor-bearing control	0	1.88 ± 0.45	6.88 ± 0.72
5-FU control	120	5.39 ± 0.42**	14.27 ± 1.65**
BGP	80	1.86 ± 0.21*	9.15 ± 0.81*
	160	3.84 ± 0.39**	11.12 ± 1.20**
	320	4.95 ± 0.63**	13.72 ± 1.06**

Compared with tumor-bearing control: *p < 0.05, ** p < 0.01. 5-FU: 5-fluorouracil.

These studies at the cell and animal levels showed that BGP has a significant tumor inhibition effect in a dose-dependent manner, which is associated with its immune enhancement function. This functional activity of BGP, which lays the foundation for its further development and applications, is related to its physicochemical properties. Thus, in-depth study of the physicochemical properties of BGP may provide a theoretical basis for the quality control measures to be adopted during batch preparation of this medicinal extract in the form of a drug.

Chemical composition and molecular weight are the most important basic physicochemical properties of BGP determined in this study. The results showed that BGP is a protein-bound polysaccharide complex, with a molecular weight of 1.46×10^5 Da.

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