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Isolation and Characterization of Immunostimulatory Polysaccharide from an Herb Tea, *Gynostemma pentaphyllum* Makino

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Water-soluble polysaccharide from *Gynostemma pentaphyllum* herb tea (PSGP) was isolated by hot-water extraction and ethanol precipitation. The chemical components and preliminary immunomodulating activity of PSGP were investigated both in vitro and in vivo. Capillary zone electrophoresis analysis showed that PSGP was a typical nonstarch heteropolysaccharide, with glucose being the main component monosaccharide (23.2%), followed by galactose (18.9%), arabinose (10.5%), rhamnose (7.7%), galacturonic acid (4.7%), xylose (3.9%), mannose (3.1%), and glucuronic acid (1.2%). PSGP could significantly stimulate peritoneal macrophages to release nitric oxide, reactive oxygen species, and tumor necrosis factor-α in a dose-dependent manner. This immunostimulating activity of PSGP was further demonstrated by its inhibition on the proliferation of human colon carcinoma HT-29 and SW-116 cells incubated with the supernatant of PSGP-stimulated macrophage culture. It is evident that PSGP is a very important ingredient responsible for at least in part the immunomodulating activity of *G. pentaphyllum* herb tea.

KEYWORDS: ***Gynostemma pentaphyllum* herb tea; polysaccharide; capillary electrophoresis; immunostimulating activity**

INTRODUCTION

The consumption of herb tea is popular in Asian countries. *Gynostemma pentaphyllum* Makino, named "Jiao-Gu-Lan" in Chinese, is a well-known herb tea in Asia. It is a saponin-rich plant and contains about 90 dammarane-type glycosides which are closely related to the component saponins in expensive ginseng, and hence, cheap *G. pentaphyllum* is regarded as "second ginseng" (*1–7*). In recent years, *G. pentaphyllum* has attracted great attention owing to its wide bioactivities for the treatments of hepatitis, hypertension, chronic bronchitis, gastritis, cancer, and other diseases (*1, 7*). For this reason, it is claimed that drinking herb tea of *G. pentaphyllum* could promote health and alleviate the severity of many disorders.

G. pentaphyllum herb tea has been produced on a large scale in China and has also been sold in Europe as an herb tea with a claim of benefit to health and beauty (*4, 8*). Traditionally, low-molecular-weight compounds, such as essential oil and saponins, have been considered as the active ingredients of this plant (*8–10*). Recently, the water-soluble polysaccharides have also been demonstrated to be partially responsible for some bioactivity of *G. pentaphyllum* herb tea (*9, 10*). In particular, these polysaccharides have greater bioavailability because they are easily soaked out into tea infusion compared with essential oil and saponins.

Interest in antitumor, immunostimulatory, and antioxidant activity of natural polysaccharides is growing (*9–11*). To the best of our knowledge, little is known about the immunomodulatory activity of polysaccharide from *G. pentaphyllum* herb tea. The present work was therefore undertaken to isolate and characterize the polysaccharide in *G. pentaphyllum* herb tea and to study its immunomodulatory activity both in vivo and in vitro.

MATERIALS AND METHODS

Materials and Reagents. The herb tea of *G. pentaphyllum* was purchased from Pingli Country Fiveleaf Gynostemma Institute, Shaanxi province, China. D-Mannose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-glucose, D-xylose, D-galactose, L-arabinose, D-fucose, and 2′,7′-dichlorofluorescein diacetate (DCF-DA) were obtained from Sigma (St. Louis, MO). Coomassie brilliant blue G250 and bovine serum albumin (BSA) were purchased from Huamei Biochemistry Co. (Shanghai, China). *m*-Hydroxydiphenyl was from Sigma Co. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), RPMI1640, phosphate-buffered saline (PBS), and fetal bovine serum (FBS) were the products of Gibco BRL (Gaithersburg, MD). Trifluoroacetic acid (TFA) was obtained from Merck (Darmstadt, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from the Beijing Reagent Plant (Beijing, China). All other chemicals were of the analytical grade available.

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Isolation of Polysaccharide from *G. pentaphyllum* (PSGP). Polysaccharide was isolated as we previously described (*12*). Briefly, the herb tea of *G. pentaphyllum* (stem and leaf) was dried at 110 °C for 2 h and mashed into a powder. The sample (500 g) was refluxed at 80 °C for 3 h in absolute alcohol. After the mixture was filtered, the residues were dried in air and then extracted in hot water (1:10, w/v) at 80 °C three times, for 1 h each time. The extracted solution was concentrated to 10% of the original volume in a rotary evaporator under reduced pressure, and then the polysaccharide was precipitated by adding 4 volumes of 95% (v/v) ethanol at 4 °C for 24 h. The sediment was collected by centrifugation (3000*g*, 10 min). Then the polysaccharide sediment was further refined by being repeatedly washed with ethanol, acetone, and ether alternately. The refined polysaccharide was dissolved in distilled water and intensively dialyzed (MW > 10 000) against distilled water, and then the retentate portion was deproteinized by a freeze−thaw process (BenchTOP, Virtis Co.) repeated eight times followed by filtration. Finally, the filtrate containing PSGP was lyophilized to yield brown water-soluble polysaccharide (7.5 g).

Physicochemical Analysis of PSGP. Moisture was determined by drying PSGP at 110 °C for 2 h and calculating the amount of evaporated water (*13*). The ash content was measured by incinerating PSGP overnight in a muffle furnace at 550 °C and weighing the residue (*13*). The total carbohydrate content in PSGP was analyzed by the phenol−sulfuric acid colorimetric method using glucose as a standard (*14*). The uronic acid content was assayed using the *m*-hydroxydiphenyl method with glucuronic acid as a standard (*15*). In addition, proteins in PSGP were quantified according to the Bradford method with BSA as a standard (*16, 17*).

The monosaccharide composition of PSGP was analyzed according to the following procedure: the samples (10 mg) were hydrolyzed in 10 mL of 2 M TFA at 110 °C for 6 h to release component monosaccharides, which were derivatized with PMP. The analysis of the PMP derivatives at a final concentration of 0.1 mg/mL was performed by capillary zone electrophoresis (CZE) as we previously described with some modification (*12*). Briefly, borate running buffers were prepared by diluting the stock solution (400 mM boric acid) to the desired concentration prior to use and adjusting the pH to the desired pH values with NaOH. MeOH in the sample solution was used as a neutral marker to measure electroosmotic flow (EOF). CZE analysis was performed on a Beckman P/ACE MDQ system. An uncoated capillary of 48.7 cm × 75 μm i.d. (effective length 38.5 cm) was used for the separation of monosaccharides, and the UV absorbance was measured at 254 nm.

Animal Experiment. Pathogen-free BALB/c mice (6−8 weeks old, 17−20 g body mass) were obtained from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). Mice were housed in plastic cages and allowed free access to water and food at 20−25 °C under a 12 h light/dark cycle at least 6 days before the experiments. The guidelines for the care of the animals were strictly followed throughout the studies. Animals were randomly divided into various groups with five mice each. PSGP was dissolved in PBS and administrated intraperitoneally to mice of experimental groups at different doses of 25, 50, and 100 mg/kg of body mass. Control animals were given the same volume of sterile PBS alone. After 8 h of treatment in vivo, murine peritoneal macrophages were isolated and analyzed (*18, 19*).

Macrophage Isolation and in Vitro Drug Treatment. Macrophages were prepared from BALB/c mice as described previously (*18*). Briefly, peritoneal macrophages were obtained from three mice. The collected cells were seeded and cultured in RPMI1640 containing 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at a density of 2×10^6 cells/well. The cells were allowed to adhere for 3 h at 37 °C in a 5% CO_2 incubator. The cultures were then washed twice with RPMI1640 to remove nonadherent cells. The adherent macrophages were treated with various designed concentrations of PSGP for 24 h. The control group received vehicle only.

Proliferation of SW-1116 and HT-29 Cells. Two colorectal adenocarcinoma cell lines, SW-1116 and HT-29, were seeded in 96-well plates at a density of 3×10^3 cells/well and allowed to adhere for 24 h in RPMI1640 containing 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were exposed to a 100 μL volume of different concentrations of PSGP or the cultured supernatants from PSGP-stimulated macrophages, while the negative controls were treated with the supernatants from unstimulated macrophages. After 6 days of culture, the cell numbers of SW-1116 and HT-29 cells were estimated using the colorimetric MTT assay as described previously (*19*). The percent viability of the treated cells was calculated as follows: (A_{570} of treated cells/A_{570} of untreated cells) × 100.

Measurement of Nitrite Release from Cells. Nitric oxide (NO) production was determined indirectly by assaying the culture supernatant for accumulated nitrite, the stable end product of NO reacted with molecular oxygen as previously described (*20*). Briefly, murine peritoneal exudate was plated onto a 96-well plate at 2×10^6 cells/well, and adhered macrophages were cultured with various concentrations of PSGP or LPS at 37 °C for 36 h. After treatment, 100 μL of isolated supernatants was allowed to react with Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride, and 2.5% phosphoric acid) at room temperature for 10 min. Nitrite products were determined by measuring the absorbance at 550 nm versus a $NaNO_2$ standard curve, and the results are shown as nanomoles per 10^6 cells.

Determination of Intracellular ROS Generation. Changes in the reactive oxygen species (ROS) level were determined by measuring the oxidative conversion of the sensitive fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA) to fluorescent 2′,7′-dichlorofluorescein (DCF) as described previously (*21*). DCFH-DA readily diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent 2′,7′-dichlorofluorescein (DCFH), which is then rapidly oxidized to form highly fluorescent DCF in the presence of ROS, and the fluorescence intensity is proportional to ROS production. Here, at the end of incubation, cells in the 96-well plate at a density of 2×10^6 per well were washed two times with PBS and then loaded with 5 μL of DCFH-DA (final concentration 10 μM in DMSO) at 37 °C for 35 min in the dark. Then the cells were resuspended with PBS. The fluorescence intensity was recorded in a fluorescence spectrophotometer (970CRT, Shanghai, China) at 485 nm excitation and 535 nm emission. Relative ROS production was expressed as a percentage of the DCF fluorescence of the control group.

Bioassay for Tumor Necrosis Factor-α (TNF-α) Secretion from Macrophages. Adhered macrophages at 2×10^6 cells/well were incubated with various concentrations of PSGP at 37 °C for 48 h. After incubation, conditioned supernatants were collected for assaying TNF-α release by determining the cytotoxicity using a bioassay of TNF-sensitive L929 cells (*22, 23*). L929 cells (3×10^5 cells, 100 μL) were cultured with serially diluted supernatants (100 μL) in the presence of actinomycin D (1 μg/mL) for 18 h in 96-well microtiter plates. The cells were washed once with PBS and stained with 0.5% crystal violet in methanol for 15 min. The plates were extensively washed with water, and the dye was extracted with methanol. The percentage of cell survival was calculated from the absorbance (*A*) values at 570 nm as follows: $A_{\text{treated}}/A_{\text{untreated control}} \times 100$.

Statistical Analysis. Data were expressed as means ± SD. Statistical differences between the treatments and the control were evaluated by Student's *t* tests. $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Physcochemical Properties of PSGP. In this study, PSGP was isolated from the traditional Chinese herb tea of *G. pentaphyllum* by hot-water extraction and ethanol precipitation. As a result, the extraction yield of PSGP could reach approximately 1.5% of the dry *G. pentaphyllum* herb tea. PSGP was easily soluble in water, but it was not soluble in organic solvents such as ethanol, ether, acetone, and chloroform. PSGP did not react with iodine−potassium iodine reagent, suggesting that PSGP was a nonstarch polysaccharide. The contents of total carbohydrate, uronic acid, protein, ash, and moisture in PSGP were 78.7%, 6.1%, 5.6%, 2.2%, and 7.9%, respectively. It was evident that the carbohydrate content of PSGP was high and

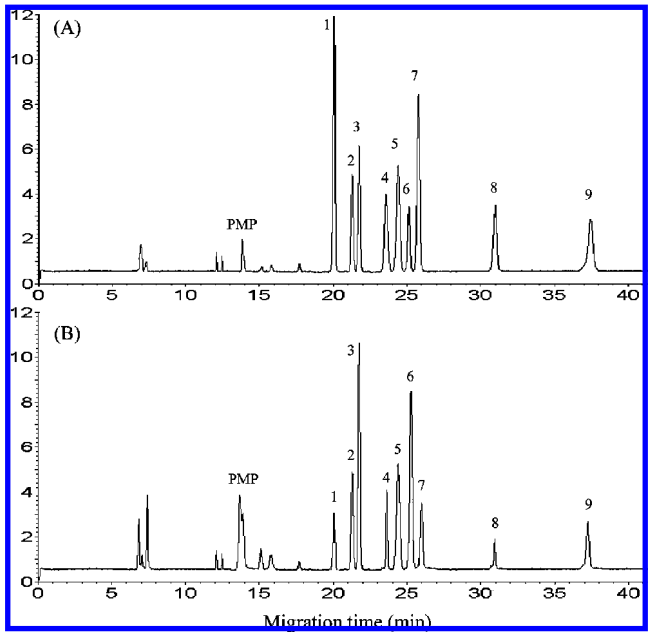

Figure 1. Typical electropherograms of the PMP derivatives of (**A**) standard aldoses and (**B**) hydrolyzed monosaccharides (g/100 g of dry sample) from the polysaccharide isolated from dry *G. pentaphyllum* herb tea (PSGP). The analysis was carried out on an unmodified fused silica capillary (58.7 cm × 75 μm i.d., effective length 48.5 cm) using 200 mM borate as the buffer at pH 11.0 (adjusted by NaOH), applied voltage 15 kV, capillary temperature 20 °C, and detection wavelength 254 nm. The peaks are identified as follows: 1, xylose; 2, arabinose; 3, glucose; 4, rhamnose; 5, fucose (internal standard); 6, galactose; 7, mannose; 8, glucuronic acid; 9, galacturonic acid.

neutral sugars were the main components. In addition, PSGP also contained a small quantity of protein because most free proteins had been removed during the isolation, especially during the freeze−thaw process. The present results suggested that PSGP was a protein-bound polysaccharide and the protein might be bound to the polysaccharide chains via electrostatic force interaction (*24*).

CZE was used to quantify the individual PMP-derivatized monosaccharide of PSGP. The separation of the PMP-derivatized monosaccharides was largely dependent on the pH and borate concentration of the running buffer. Under the optimum conditions of 200 mM borate buffer (pH 11.0), separation voltage 15 kV, and capillary temperature 20 °C, nine standard PMP-aldoses could be completely baseline separated within 38 min. The peaks in the electropherogram were identified in the order xylose, arabinose, glucose, rhamnose, fusose, galactose, mannose, glucuronic acid, and galacturonic acid (**Figure 1A**). Individual monosaccharides in PSGP were similarly separated and quantified (**Figure 1B**), demonstrating that PSGP is a typical heteropolysaccharide which consists of xylose (3.9%), arabinose (10.5%), glucose (23.2%), rhamnose (7.7%), galactose (18.9%), mannose (3.1%), glucuronic acid (1.2%), and galacturonic acid (4.7%). It should be pointed out that the CZE method could separate simultaneously a number of both neutral sugars and uronic acid in a short period of time.

Effect of PSGP on NO Release from Macrophages. NO production is associated with activated *macrophages* in the host defense mechanism (*25*). Therefore, the effect of PSGP on the NO release from murine peritoneal macrophages in vitro was investigated. As shown in **Table 1**, the treatment of the peritoneal adherent macrophages with PSGS (50, 100, 200, and 400 μg/mL) caused a significant increase in the NO production

Table 1. Effect of Polysaccharide Isolated from Dry *G. pentaphyllum* Herb Tea (PSGP) on Nitrite Release and ROS Production in Murine Peritoneal Macrophages

	treatment	nitrite release (nmol/10^6 cells)	intracellular ROS production (% of vehicle control)
in vitro[a]	vehicle (saline)	5.1 ± 0.7	100 ± 11.8
	50 μg/mL PSGP	28.5 ± 3.3[c]	122.5 ± 41.6
	100 μg/mL PSGP	43.5 ± 6.5[c]	218.8 ± 32.6[c]
	200 μg/mL PSGP	57.4 ± 7.1[c]	301.4 ± 35.5[c]
	400 μg/mL PSGP	68.8 ± 5.6[c]	329.7 ± 39.9[c]
	1 μg/mL LPS	44.9 ± 6.2[c]	291.3 ± 40.1[c]
in vivo[b]	vehicle (saline)	6.3 ± 0.8	100 ± 13.8
	25 mg/kg PSGP	29.7 ± 5.8[c]	142.9 ± 29.7[c]
	50 mg/kg PSGP	39.5 ± 7.2[c]	265.8 ± 40.2[c]
	100 mg/kg PSGP	50.4 ± 8.5[c]	318.9 ± 47.1[c]
	0.2 mg/kg LPS	51.6 ± 9.9[c]	377.4 ± 40.6[c]

[a] Peritoneal adherent cells were isolated and then treated with the indicated amount of PSGP or LPS for 36 h. The culture supernatants were subsequently isolated and analyzed for nitrite or ROS production as described in the text (mean ± SD, $n = 3$). [b] Mice were treated with the indicated amount of PSGP or LPS by intraperitoneal administration for 8 h, and peritoneal adherent cells were isolated. [c] $p < 0.05$ indicates statistically different from the control (saline) value.

from peritoneal macrophages (5.6-, 8.5-, 11.3-, and 13.5-fold, respectively). At the same time, potential macrophage activator LPS (1.0 μg/mL) increased the NO release by 8.8-fold compared with the control group ($p < 0.05$). To further confirm, BALB/c mice were treated in vivo with PSGP or LPS followed by measurement of the NO production in isolated peritoneal macrophages. As a result, the administration of PSGP (25, 50, and 100 mg/kg) or LPS (0.2 mg/kg) to mice significantly induced the NO generation in isolated peritoneal macrophages. It was concluded that PSGP possessed the potential immune-enhancing activity.

PSGP-Induced ROS Production in Macrophages. Intracellular ROS is an important indicator of immune function activation. As expected, it was found that PSGP in the range of 100−400 μg/mL significantly enhanced the intracellular ROS release in vitro in a dose-dependent manner ($p < 0.05$ versus the saline control, **Table 1**). However, no significant immunostimulating effect was observed for PSGP at the low concentration of 50 μg/mL ($p > 0.05$). It was further confirmed that intraperitoneal administration of PSGP at the concentrations of 25, 50, and 100 mg/kg to BABL/c mice also markedly promoted ROS generation in the isolated peritoneal macrophages compared with that of the control ($p < 0.05$). The results revealed that PSGP could effectively stimulate macrophages to release intracellular ROS both in vitro and in vivo.

Antitumor Activity of PSGP via Immune Stimulation. In general, the antitumor activity of plant polysaccharides is believed to be a consequence of the immune cell-mediated immune response. The present study investigated the antitumor activities of PSGP against two kinds of colorectal adenocarcinoma cell lines, HT-29 and SW-1116, in vitro. **Figure 2** shows the indirect effects of PSGP on cell proliferation of HT-29 and SW-1116 cells by a simple MTT assay. The results demonstrated that, although the polysaccharide could not directly inhibit the growth of HT-29 and SW-1116 cells in vitro, the cultured supernatant from the polysaccharide-stimulating macrophages could kill human colon cancer HT-29 and SW-1116 cells. After six days, it was observed that the treatment of human colon cancer cells with the supernatants from PSGP-stimulated macrophages caused a significant viability loss of HT-29 or SW-1116 cells in a dose-dependent manner in the concentration range 50−400 μg/mL, indicating that PSGP exerted antitumor activity by enhancing the immune function of macrophages. At

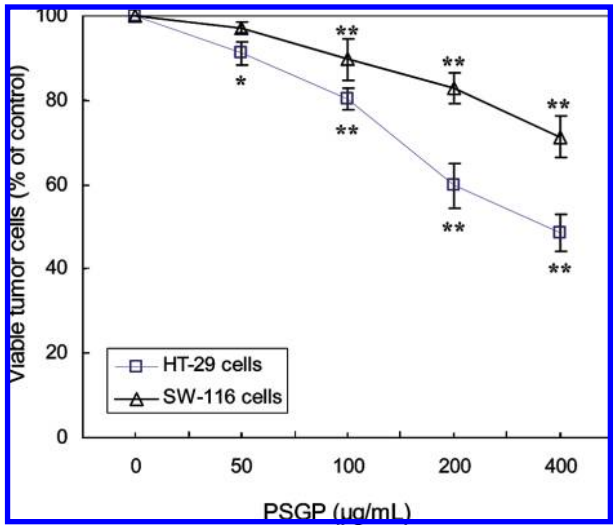

Figure 2. Indirect effect of polysaccharide isolated from dry *G. pentaphyllum* herb tea (PSGP) on the growth of HT-29 cells (□) and SW-1116 cells (△). Peritoneal adherent cells were cultured with various concentrations of PSGP for 48 h, and then HT-29 cells and SW-1116 cells on a 96-well plate were exposed to the supernatants (100 μL) from PSGP-stimulated macrophages for 6 days. The cell numbers were determined by MTT. The results are given as the relative percentage of values obtained from the controls. Values are the mean ± SD of three independent experiments. Key: *, $p < 0.05$ compared with the control group value; **, $p < 0.01$ compared with the control group value.

the same time, it was found that SW-1116 cells were less sensitive to the cytotoxicity of PSGP than HT-29 cells. For example, the colon cancer cells treated with the supernatants from 400 μg/mL PSGP-stimulated macrophages resulted in a significant increase in cytotoxicity, as reflected by a 48.4% fall in cell viability for HT-29 cells and a 71.3% fall for SW-1116 cells ($p < 0.05$) (**Figure 2**). The present result indicated that the cancer inhibitory effect of PSGP was mediated by immune stimulation.

To further confirm the indirect antitumor activity, the effect of PSGP on TNF-α secretion by macrophages was studied. In this study, adhered macrophages were cultivated in the presence or absence of PSGP for 48 h and the supernatants were collected followed by measurement of TNF-α secretion. As shown in **Figure 3**, the stimulation of macrophages with PSGP at concentrations of 50, 100, 200, and 400 μg/mL induced TNF-α release in a dose-dependent manner. To be specific, the cell viability of L929 cells was decreased by 93.7%, 85.1%, 64.7%, and 54.4%, respectively, compared with that of the control group ($p < 0.05$). The results on TNF-α secretion further proved that PSGP exhibited indirect antitumor activity.

Traditionally, consumption of herb tea made of *G. pentaphyllum* is believed to improve blood flow, eliminate toxins, and enhance the immune system (*26, 27*). In this study, we investigated the immunostimulating activity of PSGP, finding it was a potential immunostimulator which enhanced production of NO, ROS, and TNF-α. It is well-known that, when macrophages are activated, they release many effectors including NO, ROS, and TNF-α to inhibit the growth of a wide variety of microorganisms and tumor cells (*1–5*). In addition, PSGP had no direct cytotoxicity to HT-29 and SW-1116 tumor cells, but the immune-enhancing action of the polysaccharide-induced cytokine production was one of the important antitumor mechanisms.

In conclusion, *G. pentaphyllum* is a well-known edible and medicinal plant administered as an herb tea in China. Pharmacological studies of *G. pentaphyllum* extracts have illustrated a variety of health benefits, and the dammarane saponins (gypenosides or gynosaponins) are believed to be the active ingredients responsible for the biological activities of *G. pentaphyllum* (*28–30*). The present study is the first time it has been demonstrated that the polysaccharide in *G. pentaphyllum* is a typical heteropolysaccharide which possesses an immunostimulating activity.

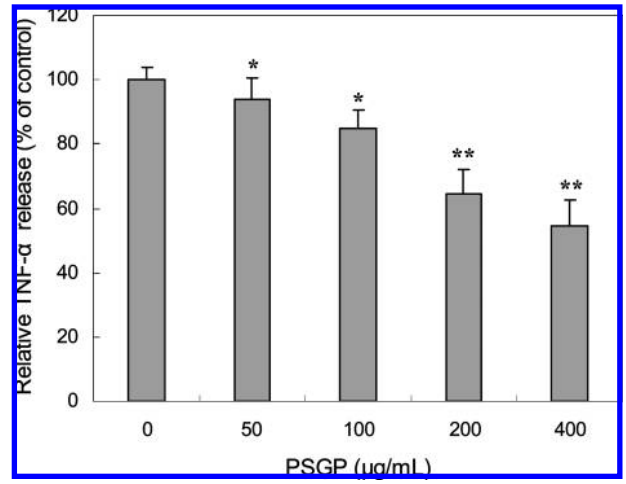

Figure 3. Effect of polysaccharide isolated from dry *G. pentaphyllum* herb tea (PSGP) on TNF-α secretion from macrophages. The relative TNF-α production in the supernatants from PSGP-stimulated macrophages was measured by a conventional bioassay using L929 cells and was expressed as a percentage of the control group value. Key: *, $p < 0.05$ compared with the control group value; **, $p < 0.01$ compared with the control group value.

LITERATURE CITED

(1) Aktan, F.; Henness, S.; Roufogalis, B. D.; Ammit, A. J. Gypenosides derived from *Gynostemma pentaphyllum* suppress NO synthesis in murine macrophages by inhibiting iNOS enzymatic activity and attenuating NF-κB-mediated iNOS protein expression. *Nitric Oxide* **2003**, *8*, 235–242.

(2) Circosta, C.; De Pasquale, R.; Occhiuto, F. Cardiovascular effects of the aqueous extract of *Gynostemma pentaphyllum* Makino. *Phytomedicine* **2005**, *12*, 638–643.

(3) Cour, B.; Molgaard, P.; Yi, Z.; La-Cour, B. Traditional Chinese medicine in treatment of hyperlipidaemia. *J. Ethnopharmacol.* **1995**, *46*, 125–129.

(4) Cui, J. F.; Eneroth, P.; Bruhn, J. G. *Gynostemma pentaphyllum*: identification of major sapogenins and differentiation from *Panax* species. *Eur. J. Pharm. Sci.* **1999**, *8*, 187–191.

(5) Huang, T. H. W.; Razmovski-Naumovski, V.; Salam, N. K.; Duke, R. K.; Tran, V. H.; Duke, C. C.; Roufogalis, B. D. A novel LXR-α activator identified from the natural product *Gynostemma pentaphyllum*. *Biochem. Pharmacol.* **2005**, *70*, 1298–1308.

(6) Lin, C. C.; Huang, P. C.; Lin, J. M. Antioxidant and hepatoprotective effects of *Anoectochillus formosanus* and *Gynostemma pentaphyllum*. *Am. J. Chin. Med.* **2000**, *28*, 87–96.

(7) Attawish, A.; Chivapat, S.; Phadungpat, S.; Bansiddhi, J.; Techadamrongsin, Y.; Mitrijit, O.; Chaorai, B.; Chavalittumrong, P. Chronic toxicity of *Gynostemma pentaphyllum*. *Fitoterapia* **2004**, *75*, 539–551.

(8) Rujjanawate, C.; Kanjanapothi, D.; Amornlerdpison, D. The anti-gastric ulcer effect of *Gynostemma pentaphyllum* Makino. *Phytomedicine* **2004**, *11*, 431–435.

(9) Cipriani, T. R.; Mellinger, C. G.; de Souza, L. M.; Baggio, C. H.; Freitas, C. S.; Marques, M. C. A.; Gorin, P. A. J.; Sassaki, G. L.; Iacomini, M. A polysaccharide from a tea (infusion) of *Maytenus ilicifolia* leaves with anti-ulcer protective effects. *J. Nat. Prod.* **2006**, *69*, 1018–1021.

(10) Wang, Z. J.; Luo, D. H. Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum* Makino. *Carbohydr. Polym.* **2007**, *68*, 54–58.
(11) Chen, H. X.; Zhang, M.; Xie, B. J. Quantification of uronic acids in tea polysaccharide conjugates and their antioxidant properties. *J. Agric. Food Chem.* **2004**, *52*, 3333–3336.
(12) Yang, X. B.; Zhao, Y.; Lv, Y. Chemical composition and antioxidant activity of an acidic polysaccharide extracted from *Cucurbita moschata* Duchesne ex Poiret. *J. Agric. Food Chem.* **2007**, *55*, 4684–4690.
(13) Rodriego-Otero, J. L.; Paseiro, P.; Simal, J.; Cepeda, A. Mineral content of the honeys produced in Galicia (north-west Spain). *Food Chem.* **1994**, *49*, 169–171.
(14) Dubois, M.; Gilles, K. A.; Haamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
(15) Filisetti-Cozzi, T. M. C. C.; Carpita, N. C. Measurement of uronic acids without interference from neutral sugars. *Anal. Biochem.* **1991**, *197*, 157–162.
(16) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(17) Yang, X. B.; Zhao, Y.; Lv, Y. *In vivo* macrophage activation and physicochemical property of the different polysaccharide fractions purified from *Angelica sinensis*. *Carbohydr. Polym.* **2008**, *71*, 372–379.
(18) Kim, G. Y.; Choi, G. S.; Lee, S. H.; Park, Y. M. Acidic polysaccharide isolated from *Phellinus linteus* enhances through the up-regulation of nitric oxide and tumor necrosis factor-α from peritoneal macrophages. *J. Ethnopharmacol.* **2004**, *95*, 69–76.
(19) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63.
(20) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. Analysis of nitrate in biological fluids. *Anal. Biochem.* **1982**, *126*, 131–138.
(21) Wang, H.; Joseph, J. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biol. Med.* **1999**, *27*, 612–616.
(22) Ando, I.; Tsukumo, Y.; Wakabayashi, T.; Akashi, S.; Miyake, K.; Kataoka, T.; Nagai, K. Safflower polysaccharides activate the transcription factor NF-kappa B via Toll-like receptor 4 and induce cytokine production by macrophages. *Int. Immunopharmacol.* **2002**, *2*, 1155–1162.
(23) Ferrari, M.; Fornasiero, M. C.; Isetta, A. M. MTT colorimetric assay for testing macrophage cytotoxic activity *in vitro*. *J. Immunol. Methods* **1990**, *131*, 165–172.
(24) Roudsari, M.; Nakamura, A.; Smith, A.; Corredig, M. Stabilizing behavior of soy soluble polysaccharide or high methoxyl pectin in soy protein isolate emulsions at low pH. *J. Agric. Food Chem.* **2006**, *54*, 1434–1441.
(25) Jeong, S. C.; Jeong, Y. T.; Yang, B. K.; Song, C. H. Chemical characteristics and immuno-stimulating properties of biopolymers extracted from *Acanthopanax sessiliflorus*. *J. Biochem. Mol. Biol.* **2006**, *39*, 84–90.
(26) Weisburger, J. H. Tea and health: ahistorial perspective. *Cancer Lett.* **1997**, *114*, 315–317.
(27) Monobe, M.; Ema, K.; Kato, F.; Maeda-Yamamoto, M. Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *J. Agric. Food Chem.* **2008**, *56*, 1423–1427.
(28) Wang, Q. F.; Chiang, C. W.; Wu, C. C.; Cheng, C. C.; Hsieh, S. J.; Chen, J. C.; Hsieh, Y. C.; Hsu, S. L. Gypenosides induce apoptosis in human hepatoma Huh-7 cells through a calcium/reactive oxygen species-dependent mitochondrial pathway. *Planta Med.* **2007**, *73*, 535–544.
(29) Qian, X. H.; Wang, Y. X.; Tang, X. L. Effects of *Gynostemma pentaphllum* Makino polysaccharide (PGP) on immunefunction. *J. China Pharm. Univ.* **1998**, *30*, 51–53.
(30) Tanner, M. A.; Bu, X.; Steimle, J. A.; Myers, P. R. The direct release of nitric oxide by gypenosides derived from the herb *Gynostemma pentaphyllum*. *Nitric Oxide* **1999**, *3*, 359–365.

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