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Full Length Research Paper

Immunomodulatory and antioxidant activity of a *Siraitia grosvenorii* polysaccharide in mice

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The aim of this present study was to investigate the immunomodulatory and antioxidant activities of *Siraitia grosvenorii* polysaccharide (SGP) using a mouse model. The roles of regulation of SGP were investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, fluorescence method and ELISA method. The chemical structures were analyzed by Fourier transform infrared spectroscopy (FTIR) and gas chromatography (GC). The results show that SGP promoted the proliferation of spleen cells and regulated the level of reactive oxygen species (ROS) *in vitro*. Furthermore, it significantly raised spleen and thymus indices and superoxidase dismutase activity and regulated the cytokine levels of spleen and thymus in mice *in vivo*. These findings suggest that SGP possesses immunomodulatory and antioxidant effects.

Key words: *Siraitia grosvenorii* polysaccharide (SGP), cell proliferation, reactive oxygen species, superoxidase dismutase, cytokines.

INTRODUCTION

Polysaccharides, as an important kind of carbohydrates in nature mainly from different plant sources, have been demonstrated to have a variety of chemical and biological activities both *in vivo* and *in vitro,* such as anti-tumor (Peng et al., 2005, 2003; Wang et al., 2010), antivirus (Huang et al., 2008), anti-inflammation (Pereira et al., 2000; Silva and Parente, 2001), anticancer (Shi et al., 2007; Umemura et al., 2003), anticoagulant hypo-glycemic (Veena et al., 2007) and immunological activities (Shao et al., 2004; Maisuthisakul and Gordon, 2009), which made them possible to be used in many clinical applications.

Siraitia grosvenorii (Swingle), known as Luo Han Guo in China, a perennial vine of the Cucurbitaceae family (Lu An-min, 1986), has been cultivated in Guangxi Province of China as natural sweetener and traditional medicinal herb. In recent years, the studies have shown that the extract from *S. grosvenorii* possess properties of immune enhancement, anti-oxidation, anti-diabetes and anti-inflammation both *in vitro* and *in vivo*, and was also used as a substitute sugar for obese and diabetic patients (Zhang et al., 2006; Song et al., 2006, 2007; Pan et al., 2009). Sugar composition analysis by high performance liquid chromatography (HPLC) indicated that *Siraitia grosvenorii* polysaccharide (SGP) was composed primarily of D-glucose, D-galactose, D-xylose, L-arabiose, L-rhamnose and glycuronic acid (Li et al., 2005,2007). Relative molecular mass of SGPS1 and SGPS2 were 430 and 650 kD respectively (Li et al., 2005).

It was reported that SGP evidently improved macrophages phagocyte percentage and phagocytic index, raised the levels of serum hemolysin and promoted lymphocyte transformation rate in mice (Li et al., 2008). *M. grosvenori* Swingle extract down regulated inflammatory iNOS and COX-2 gene expression in macrophages by inhibiting the activation of NF-κB and by interfering with the activation of PI3K/Akt/IKK and MAPK (Pan et al., 2009).

The objective of this study was to investigate the possible modulatory effect of SGP on immunity and antioxidant activity using a mouse model by different analytical methods.

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MATERIALS AND METHODS

Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and concanavalin A (ConA), lipopolysaccharide (LPS), were purchased from Sigma Chemical Co., Saint Louis, USA; RPMI-1640 medium was obtained from Gibco Laboratories, USA; fetal calf serum (FCS) was obtained from Hyclone, South America; reactive oxygen species (ROS) assay kit was from PULILAIJIYIN C1300, Beijing, China; IL-1 detecting ELISA kit and IL-2 detecting ELISA kit were from ADL Co., USA. All other chemicals and solvents used were of analytical grade.

Extraction, isolation and chemical characterization of SGP

Siraitia grosvenorii (Swingle) is commercially available in Guangxi, China. Dried *S. grosvenorii* (300 g) was cut into small pieces and powdered. It was extracted with 3.6 L of distilled water at 100°C each 1.5 h for two times, filtered through four sheets of gauze and centrifuged at 3 000 *g* for 10 min to collect water-insoluble materials. The aqueous extract was concentrated to 300 ml by evaporation at 100°C and treated with six volumes of 95% ethanol at 4°C for overnight. The obtained extraction was centrifuged at 664 *g* for 20 min. Precipitate was washed with 100% ethanol once and lyophilized to get a light brown powder (neutral and water-soluble polysaccharide).The output of the polysaccharide was 6.89% (w/w). SGP was dissolved in physiological saline solution (0.9% w/v), filtered through a 0.22 μm filter and stored at 4°C before use.

FT-IR spectroscopy and sugar composition analysis

Infrared spectra of polysaccharides were recorded on a FTIR-8400S spectrophotometer (SHIMADZU, Co.). Samples were dried at 35 to 44°C in vacuum over P_2O_5 for 48 h prior to making pellet with KBr powder. For monosaccharide composition analysis, SGP samples were hydrolyzed at 100°C for 6 h with 1 mol/L H_2SO_4, and the resulting samples after vacuum drying were added with hydroxylamine hydrochloride amine. Internal standard content and pyridine was set in water-bath for 30 min and shaken every other 10 min, and then acetic anhydride was mixed in water at 90°C bath for another 30 min to allow acetylization. The end product decompressed to dryness was dissolved with trichloromethane and detected by gas chromatograph (Agilent Technologies 6890 N, DB-WAX SQC column, 0.25 mm× 30 m× 0.25 μm, Network GC System). The temperature program was set to increase at an increment of 8°C/min from 120 to 250°C with N_2 as the carrier gas. The standard monosaccharides were measured following the same procedure as described earlier.

Preparation of spleen cells suspension

Murine spleens were aseptically excised from mice and placed in a phosphate buffered saline (PBS, pH 7.2). The spleens were cut into small pieces and squeezed with a syringe plunger to extrude cells. The cell suspension was centrifuged at 73 *g* for 5 min at room temperature. The cell pellet was resuspended in 10 ml of lysing buffer (0.16 M NH_4Cl and 0.17 M Tris, pH 7.2) and incubated in ice for 5 min to lyse the red blood cells. The resulting cell pellet was washed twice with PBS and resuspended in RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mmol L^{-1} L-glutamine, 25 μmol L^{-1} 2-mercaptoethanol, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin sulfate. Cell viability was determined using Trypan blue dye exclusion method. To remove adherent cells, such as macrophages, the spleen cells were incubated for 1 h in culture flask at 1.0×10^6 cells/ml.

Proliferation of spleen cells

Proliferation of spleen cells was measured by MTT assay (Colleselli et al., 2006). Briefly, the splenic cells were adjusted to 1.0×10^6 cells/ml and transferred to 96-well tissue culture plates in the absence or presence of 5 μg/ml of Con A or 10 μg/ml of LPS and SGP at concentrations of 25 to 200 μg/ml. The cells were incubated for 72 h at 37°C in a humidified atmosphere of 5% CO_2. 20 μl of MTT solution (5 μg/ml) was added to spleen cell solution before the final 4 h incubation. After incubation, supernatant was removed and the formazan product in the cells was dissolved by the addition of 100 μl dimethylsulfoxide (DMSO) to each well. Plates were shaken for 10 min and absorbance was measured at 570 nm using a plate reader (TECAN A-5082, Austria).

Measurement of ROS generation

The intracellular ROS production was measured using reactive oxygen species assay kit according to the manufacturer's instruction (PULILAIJIYIN C1300, Beijing, China). DCFH-DA penetrates the cells and is hydrolyzed by intracellular esterase to the non-fluorescent DCFH, which can be rapidly oxidized to the highly fluorescent 2, 7-dichlorofluorescein (DCF) in the presence of ROS. Briefly, the splenic cells at 1×10^6 cells/ml were treated with SGP at 0 (normal control), 25, 50,100 and 200 μg/ml at 37°C for 4 h, after which they were suspended in PBS. Then DCFH-DA was added at a final concentration of 10 μmol/L for additional 40 min at 37°C in the dark. The cells were washed twice and then resuspended in PBS. The fluorescent intensity of the cell suspensions was then detected with a fluorescence plate reader measured at 535 nm with excitation wavelength at 485 nm (WALLAC VICTOR^2 1420-012, Finland).

In vivo experiment

50 BALB/c inbred mice (8 weeks old, 18 to 20 g) were provided by the Animal Center of Guangxi Traditional Chinese Medicine Institute, Guangxi, China. The animals were kept under normal laboratory conditions at room temperature and allowed free access to standard laboratory pellet diet and water during the experiment. Animal ethics guidelines of Institutional Animal Ethics Committee were followed. The animals were randomly divided into five groups and 10 mice in each group. Group 1 was normal control and the animals were treated only with physiological saline solution (0.9% w/v).

In the treatment groups, the mice were administered with SGP at doses of 50, 100, 200 and 400 mg/kg body weight by intraperitoneal injection respectively once every 2 days for 7 days. On Day 15, the animals were weighed and then killed by decapitation. The spleens and thymus of the mice were immediately removed, weighed and kept on ice and their homogenates were made in ice-cold isotonic physiological saline. The homogenates were centrifuged at 10 000 *g* for 20 min to collect supernatants to measure the superoxidase dismutase activity (SOD) activity and nitric oxide (NO) level with spectrophotometer or ELISA methods.

Effects of SGP on spleen and thymus indices in mice

The spleen and thymus indices were calculated according to the following formula:

Spleen or thymus index (mg/g) = weight of spleen or thymus /body weight

NO level assay

The NO level in the homogenates of both thymus and spleen was measured by the method of Griess assay (Chauhan et al., 2009; Schepetkin et al., 2008). Briefly, the supernatant of the homogenate (100 µl) of thymus or spleen was incubated with an equal volume of Griess reagent [one part of 0.1% (w/v) naphthylethylenediamine dihydrochloride in distilled water mixed together with one part of 1% (w/v) sulphanilamide (p-aminobenzenesulfonic acid) in 5% (v/v) H_3PO_4] at room temperature for 10 min. The absorbance was measured at 540 nm using an automatic plate reader (TECAN A-5082, Austria). The NO concentration was determined by a standard curve of the sodium nitrite ($NaNO_2$). The concentration of nitric oxide was expressed as µmol /L.

Measurement of SOD activity

The activity of SOD was analyzed by the auto-oxidation of pyrogallol with some modifications (Matsumura et al., 2003).The spleen or thymus homogenate (10 µl) was mixed with 4.5 ml of 0.05 mol/L phosphate buffer (pH 8.3). The reaction mixture was incubated at 25°C for 25 min, and then 10 µl of pyrogallol (50 mmol/L) was added. The absorbance at 325 nm was measured against blank samples every 30 s. SOD activity was calculated as:

$$d = [(\triangle A_{325}-0.07)/0.07\times 100\%]/50\%\times V_1/(V_2\times V_3)\times \text{sample dilution multiple}$$

Where $\triangle A_{325}$ is the absorbance variation value every one minute of the reaction mixture, V_1 is the total volume of reaction mixture, V_2 is the volume of activity unit definition and V_3 is the volume of the test solution.

Determination of IL-1 and IL-2 level

Goat anti-mouse Interleukins1 and 2 enzyme-linked immunosorbent assay (ELISA) kits (Adlitteram Diagnostic Laboratories, USA) were used to detect IL-1 or IL-2 level in the supernatant of the homogenates of both thymus and spleen. The concentrations of cytokines were determined by extrapolation from IL-1 or IL-2 standard curves according to the manufacturer's protocol.

Evaluation of hydroxyl radical (•OH) scavenging assay

The •OH scavenging activity was investigated following the published method with a minor modification (Pang et al., 2000). Orthophenanthroline (5 mmol/L, 0.6 ml) was mixed with 1.6 ml of reaction buffer phosphate buffer (0.75 mol/L, pH 7.4) and 0.2 ml of ferrous sulphate (7.5 mmol/L). The reaction solution was incubated for 60 min at 37°C and then SGP solution (50, 100, 200 and 400 µg/ml), 0.2 ml H_2O_2 (1%) and distilled water was added to the mixture. The absorbance of the mixture was measured at 536 nm. Distilled water was used as a blank control. The •OH scavenging activity was calculated as:

$$d= [(A_1-A_2)/ (A_3-A_0)] \times 100\%$$

Where, A_0 is the absorbance of the blank control, A_1 is the absorbance of the presence of both the polysaccharide and H_2O_2, A_2 is the absorbance of H_2O_2 alone and A_3 is the absorbance of both the absence of the polysaccharide and H_2O_2.

Statistical analysis

The data were expressed as the mean ± SD and analyzed via ANOVA and Duncan's multiple range tests using SPSS/11.5 software. $P < 0.05$ was considered statistically significant, and $P < 0.01$ and $P < 0.001$, highly significant.

RESULTS

Compositional analysis of the polysaccharides

As shown in Figure 1, bands at 3600 to 3200 cm^{-1} were the characteristic of the O–H stretching vibration due to existence of hydrogen bond between intramolecular and intermolecular interactions according to FTIR spectra for SGP. The bands at 3000 to 2800 cm^{-1} were the C–H bending vibration of the methyl group and are characteristic of the C–H stretching vibration. The strong bands at 1030 to 1190 cm^{-1} were due to the C = O stretching vibration of the carbonyl group, which caused C-O-H and C-O-C, respectively. The weak peaks at 1200 to 1400 cm^{-1} were ascribed to the C–H stretching vibration of the variable angle. The bands at neighbouring 1648 cm^{-1} were the CH_3CONH-amid characteristic.

In addition, bands at 844 ± 8 cm^{-1} confirmed the characteristic of pyranose α-type C-H variable angle vibration. The absorption peaks and stretching vibration were distinctive configuration of polysaccharides.

Sugar composition analysis by gas chromatography indicated that SGP was composed primarily of D-glucose, D-galactose, D-arabinose, and L-rhamnose monohydrate with molar ratio of 5.98: 0.77: 0.38: 0.12 (Figure 2).

ROS generation

The intracellular ROS level in murine spleen cells was detected using a fluorescent probe, 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA). As shown in Table 1, when the cells were cultured in the presence of 25, 50, 100 and 200 µg/ml of SGP, the production of ROS decreased. Particularly, SGP at 25 and 50 µg/ml significantly decreased the intracellular ROS level as compared to the normal control ($P < 0.01$).

SGP promoted proliferation of murine splenic cells *in vitro*

The splenic cells in different treatment groups were determined by MTT assay (Table 2).The results showed that SGP at 12.5 to 200 µg/ml significantly promoted the proliferation of splenic lymphocytes compared with the normal control ($P < 0.05$ to 0.01). Moreover, SGP (12.5 to 200 µg/ml) with Con A increased proliferation of the

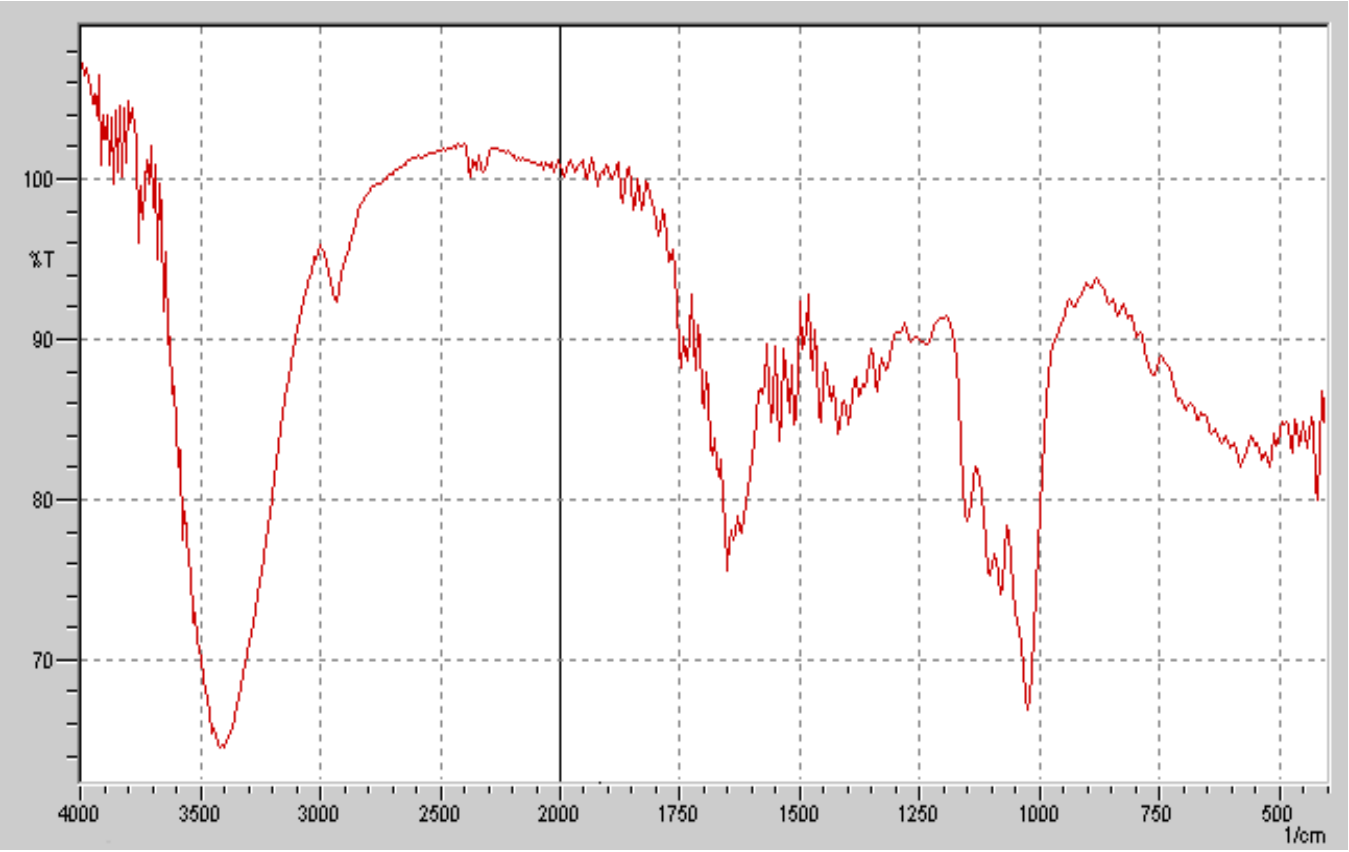

Figure 1. The FTIR spectra of water-soluble polysaccharide from the *Siraitia grosvenori.*

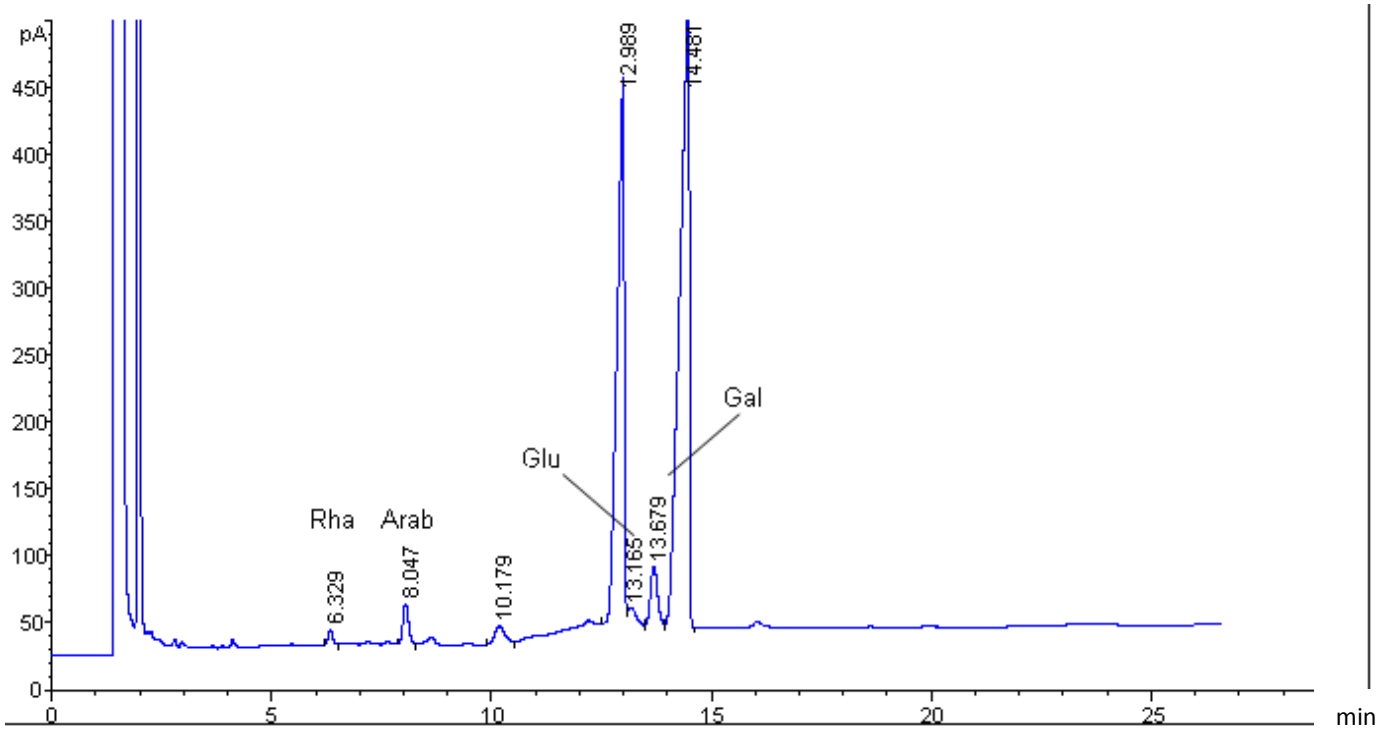

Figure 2. GC spectra of SGP.

Table 1. Effect of SGP in different concentration on ROS production.

Concentration of SGP (μg/ml)	Fluorescent intensity (OD)
0	66440±16563.72
25	38443±4202.14**
50	39649.67±2692.87**
100	53671±3038.62
200	55855±6740.48

**p < 0.01 vs. control group; n = 3.

Table 2. SGP promoted murine spleen cells proliferation *in vitro.*

SGP (μg/ml)	SGP	SGP+ Con A (5 μg/ml)	SGP+LPS (10 μg/ml)
0	0.463±0.013	—	—
0	—	0.561±0.011	0.575±0.024
12.5	0.500±0.027**	0.581±0.034	0.569±.0.60
25	0.497±0.016**	0.625±0.047###	0.653±0.049△△
50	0.486±0.018*	0.590±0.021	0.636±0.014△△
100	0.491±0.009*	0.567±0.011	0.599±0.018
200	0.498±0.012**	0.574±0.013	

*p < 0.05 and **p < 0.01 vs. untreated control; ###p < 0.001 vs Con A alone; △△p < 0.01 vs. LPS treatment alone; n = 6.

Table 3. Effect of SGP on spleen and thymus indices in mice.

SGP (mg/kg)	Spleen index (mg/g)	Thymus index (mg/g)
0	5.57±1.13	2.95±0.42
50	5.85±1.24	3.29±0.76
100	6.87±1.08*	2.76±0.70
200	7.30±1.53**	3.51±0.70
400	5.39±0.78	3.76±0.76*

*p < 0.05; **p < 0.01 vs normal control; n = 10.

splenic T-cells and the largest effect was attributed to the group treated with 25 μg/ml (P < 0.001) as compared with Con A (5 μg/ml) treatment alone.

In the presence of LPS (10 μg/ml), SGP at 25 and 50 μg/ml markedly promoted the proliferation of the splenic B-cells (P < 0.01) when compared with LPS (10 μg/ml) alone. These results suggest that the polysaccharide from *S. grosvenorii* promoted the proliferation of both T and B-cells in mice.

Effects of SGP on spleen and thymus indices in mice

The spleen and thymus indices of the animals treated with SGP were raised when compared with the normal control (Table 3). SGP markedly raised the spleen index (P < 0.05-0.01) at the dose of 100 and 200 mg/kg body weight, and significantly increased thymus index at 400 mg/kg body weight (P < 0.05).

Effects of SGP on NO level and SOD activity

NO content of both spleen and thymus were elevated when the animals were administered with SGP by intraperitoneal injection (Table 4). The NO produced by the spleen or thymus respectively reached a peak at the dose of 50 mg/kg bodyweight (P < 0.05) and 100 mg/kg bodyweight (P < 0.001). SGP increased the production of NO in splenic and thymus cells. Table 5 illustrates the effect of SGP on the SOD activity in mice. SGP markedly raised the activity of SOD (P < 0.01 to P < 0.001) in a dose dependent manner in both thymus and spleen of mice.

Table 4. Effect of SGP on NO production in murine thymus and spleen.

SGP (mg/kg)	Spleen NO (μ mol/L)	Thymus NO (μ mol/L)
0	36.63±12.38	14.14±4.17
50	56.65±22.77*	17.43±3.39
100	42.27±12.40	54.42±16.84***
200	45.03±27.71	18.38±5.59
400	53.06±16.66	17.67±5.86

*p < 0.05; **p < 0.01 vs normal control; n = 10.

Table 5. Effect of SGP on the activity of SOD in murine thymus and spleen.

SGP (mg/kg)	Spleen SOD b (units/ml)	Thymus SOD (units/ml)
0	130.84±35.13	213.13±92.67
50	168.76±25.01***	227.23±81.29
100	216.68±39.51***	319.33±88.81**
200	248.91±39.36***	341.85±56.05**
400	187.31±29.23**	388.59±60.80***

*p < 0.05; ***p < 0.01 vs normal control; n = 10.

Table 6. Effect of SGP on IL-1 Level in murine thymus and spleen.

SGP (mg/kg)	Speen IL-1 (pg/ml)	Thymus IL-1 (pg/ml)
0	39.92±3.74	34.08±1.57
50	38.17±6.30	33.82±1.60
100	42.76±6.95	34.17±2.64
200	39.60±1.95	32.48±2.73
400	37.72±4.47	31.81±1.57

*p < 0.05 vs normal control; n = 10.

Table 7. Effect of SGP on IL-2 production in murine thymus and spleen.

SGP (mg/kg)	SpleenIL-2 (pg/ml)	Thymus IL-2 (pg/ml)
0	24.83±7.97	20.36±0.77
50	40.51±16.52*	20.58±3.45
100	27.11±7.82	23.03±4.70
200	27.09±6.09	19.62±2.14
400	33.69±14.99	20.87±7.29

*p < 0.05 vs normal control; n = 10.

Bioassay of IL-1 and IL-2 levels

Administration of SGP showed a significant increase in IL-2 level at 50 mg/kg ($P < 0.05$) in spleen and a significant decrease in IL-1 level at 400 mg/kg ($P < 0.05$) in thymus when compared to normal control respectively (Tables 6 and 7).

Effect of SGP on hydroxyl radical scavenging activity

The hydroxyl radical belongs to one of the representative reactive oxygen species generated in the process of most diseases. In this study, the radical-scavenging activity was enhanced in accompany with the increased concentration (50, 100, 200 or 400 μg/ml). The

Table 8. Hydroxyl radical-scavenging activity of SGP *in vitro.*

1% SGP (ml)	Final concentration of SGP (μg/ml)	Hydroxyl radical-scavenging effect (%)
0.3	50	36.23±4.10
0.6	100	39.72±6.68
1.2	200	42.77±3.14
2.4	400	51.18±1.98**

*p < 0.05 vs normal control; n = 3.

polysaccharide at 400 μg/ml showed stronger antioxidant activities (P < 0.01) than that with 50 μg/ml. The effect of SGP on the radical-scavenging activity showed a dose-dependent increase (Table 8).

DISCUSSION

In recent years, polysaccharides have attracted increasing attention because of their effective antioxidant activity. Many studies have shown that polysaccharides improved the activity of antioxidant enzymes, scavenged free radicals, and inhibit lipid oxidation (Sun et al., 2009; Li et al., 2006; Matsumura et al., 2003). S*. grosvenorii* (SG), a traditional medicinal herb in China used as a substitute sugar for obese and diabetic patients, caused an enhanced immunity (Song et al., 2006).

One of the most important defense systems of neutrophils corresponds to their ability to mediate a strong oxidative burst through the formation of reactive oxygen species (ROS) (Freitas M et al., 2009). In a normal healthy cell, equilibrium between the generation of ROS and their elimination by the antioxidant system is maintained. ROS is a key regulator of homeostasis, whereas the increased generation of ROS plays an important role in inflammation, host defense response and tissue repair (Girard-Lalancette et al., 2009). The molecules including superoxide, hydroxyl radicals, nitric oxide (NO), and hydrogen peroxide commonly have been designated as ROS (McArdle et al., 2005). In addition, ROS was shown to be responsible for the inducible expression of the genes associated with inflammatory and immune responses (Camins et al., 1999). Using ROS-sensitive fluorescence dye, DCFH, we found that SGP significantly down-regulated the intracellular ROS level as compared to the normal control group at 25 and 50 μg/ml (P < 0.01). The results indicate that SGP regulated the intracellular ROS generation in spleen cells *in vitro*.

Some polysaccharides have been reported to exhibit obvious immune modulatory activity. For example, *Strongylocentrotus nudus* egg polysaccharide (SEP) stimulated the proliferation of T lymphocytes in a dose-dependent manner (Liu et al., 2008). Polysaccharides from *Phascolosoma esulenta* could significantly raise spleen and thymus indices and enhance Con A-stimulated spleen cells proliferation in mice (RenJie, 2008). In this experiment, the LPS or Con A was chosen as B lymphocytes or T lymphocytes stimulator. The results show that SGP could significantly activate both B lymphocytes and T lymphocytes to enhance immunity-stimulating activities.

Nitric oxide is one of the smallest and most diffusible signal molecules known (Moroz and Kohn, 2007; Lim et al., 20019) and also is a very active molecule involved in many and diverse biological pathways where it has proved to be protective against damages provoked by oxidative stress conditions (García-Mata and Lamattina, 2001). Nitric oxide can have both positive and negative effects depending on the concentrations achieved in the diseases (Hu and Brindle, 2005). In the study, the result show that SGP was able to increase NO production in the spleen in mouse. The rise of NO level occurred in parallel with the rise of that in thymus. From this point of view, SGP may modulate immune function via regulating the level of NO in immune organs.

Antioxidant enzymes are considered to be a primary defense that prevents biological macromolecules from damage in antioxidant systems (Chen et al., 2007). SOD protects cells against O_2^- by dismutation of the highly reactively superoxide anion and to a less reactive species, H_2O_2 plus O_2 (Yu et al., 2009). H_2O_2 can be eliminated by glutathione peroxidase or catalase (Chiou and Tzeng, 2000; Hseu et al., 2008; Caldefie-Chézet et al., 2002). Among the oxygen radicals, the hydroxyl radical is the most reactive and severely damages adjacent biomolecules (Sakanaka and Ishihara, 2008). *Ganoderma lucidum* polysaccharides could significantly enhance the antioxidant enzyme activities (SOD, CAT and GPx) to maintain the balance of the pro-oxidant/antioxidant inside the body (Chen et al., 2009). Thus, SOD is one of the essential cofactors for antioxidants that protects against endogenous oxygen radicals generated during pathogenic processes and other changes in the physiological state. In this study, SOD activity was significantly increased in mice treated with SGP when compared with the normal control *in vivo*. This result is coincident with decreased level of ROS *in vitro*. Furthermore, SGP enhanced hydroxyl radical-scavenging activity *in vitro* in a dose-dependent manner.

These results indicate that SGP had antioxidant activities. IL-1 is a pleiotropic cytokine and a major messenger between the immune systems and the neuroendocrine. Various enzymes, including the IL-1β converting enzyme (ICE), can activate pro-IL-1 stored in the cytoplasm of various cells when coordinates host response to diverse stimuli, including infection, immunological and inflammatory processes and endogenous mediators of cell and tissue injury (Borish and Steinke, 2003; Verri et al., 2006). IL-1 mediates multiple biological effects, including cytotoxic activity against several types of tumor cell and the enhancement of antibody production by B-lymphocytes (Sonoda et al., 1998). Interleukin-2 (IL-2) is a type of immunoregulatory cytokines and it is instrumental in the organism's natural response to microbial infection and in discriminating between foreign (non-self) and self (Chiou and Tzeng, 2000). The road paved by intermittent IL-2 therapy sets the stage for testing more immunological compounds as well as the strategies of manipulation of the immune system against the infection (Alfano and Poli , 2005). IL-2 is produced by T lymphocytes in the course of T cell activation and because IL-2 promotes and regulates the growth and function of immune cells (Pahlavani and Richardson, 1996).In this study, we found that *S. grosvenorii* polysaccharide significantly induced an increase in the level of IL-2 at 50 µg/ml and down-regulated the secretion of IL-1β in thymus at 400 µg/ml. The findings indicate that SGP stimulated the immunity activity of T lymphocytes and B lymphocytes by inducing IL-2 production, which in turn reduced IL-1β release in mice. Our experimental data support the results described by Hu and Zheng (2004).

Conclusion

Our results show that SGP, a novel polysaccharide isolated from the *S. grosvenorii*, had modest but statistically significant immunoregulation actions. SGP significantly enhanced both spleen and thymus indices in mice *in vivo*, and stimulated Con A/LPS-induced splenocyte proliferation *in vitro*. SGP alone stimulated T lymphocytes and B lymphocytes proliferation and regulated secretion of both IL-1 and IL-2 in mice to enhance the immune and anti-inflammatory activity.

Our data indicated that SGP promoted production of nitric oxide and enhanced SOD activity in spleen and thymus of mice. In addition, SGP regulated the level of ROS and enhanced hydroxyl radical-scavenging activity *in vitro*. This suggested that SGP treatment had antioxidant effect, at least in part, from the activation of SOD and regulation of ROS. Thus, this study may provide a basis for the use of SGP as a strong potential immunostimulant for the food and pharmaceutical industries, and the immune-modulating activity of SGP should be explored in further studies.

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