Effects of Siraitia grosvenorii Polysaccharides on Immune Function in Cyclophosphamide-Induced Immunosuppressed Mice: Postprint

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Abstract

To investigate the effects of Siraitia grosvenorii polysaccharide on the immune function of immunosuppressed mice, experimental mice were randomly divided into a normal group, a model group, low-, medium-, and high-dose Siraitia grosvenorii polysaccharide groups (25 mg • kg-1, 50 mg • kg-1, 100 mg • kg-1), and a levamisole group. An immunosuppressed mouse model was established via intraperitoneal injection of cyclophosphamide (20 mg • kg-1). Following 14 consecutive days of intragastric administration, the immune organ index, clearance index (K), phagocytic index ( ), T and B lymphocyte proliferation levels, ear swelling degree, half hemolysis value (HC50), and contents of immunoglobulin G (IgG), immunoglobulin M (IgM), IL-2, IL-4, IL-6, and TNF- were measured in each group, and spleen tissue pathomorphological changes were observed to evaluate the effects of Siraitia grosvenorii polysaccharide on the immune function of immunosuppressed mice. The experimental results demonstrated that all dose groups of Siraitia grosvenorii polysaccharide (25 mg • kg-1, 50 mg • kg-1, 100 mg • kg-1) significantly increased the immune organ index, half hemolysis value (HC50), and B lymphocyte proliferation capacity; markedly decreased ear swelling degree; and significantly increased the contents of IgG, IgM, IL-2, IL-4, IL-6, and TNF- in immunosuppressed mice. Siraitia grosvenorii polysaccharide dose groups (50 mg • kg-1, 100 mg • kg-1) significantly enhanced T lymphocyte proliferation capacity and markedly increased the clearance index (K) and phagocytic index ( ). Histopathological observation of spleen tissue revealed that Siraitia grosvenorii polysaccharide could alleviate pathological damage to the spleen in immunosuppressed mice. These findings indicate that Siraitia grosvenorii polysaccharide can significantly enhance the immune function of cyclophosphamide-induced immunosuppressed mice.

Full Text

Effects of Siraitia grosvenorii Polysaccharides on Immune Function in Cyclophosphamide-Induced Immunosuppressed Mice

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Abstract

This study investigated the immunomodulatory effects of Siraitia grosvenorii polysaccharides (SGP) on immunosuppressed mice. Kunming mice were randomly divided into six groups: normal control, model control, SGP low-dose (25 mg · kg ^{1}), medium-dose (50 mg · kg ^{1}), high-dose (100 mg · kg ^{1}), and levamisole positive control groups. An immunosuppressive mouse model was established by intraperitoneal injection of cyclophosphamide (20 mg · kg ^{1}). After 14 consecutive days of intragastric administration, various immune parameters were measured including immune organ indices, carbon clearance index (K), phagocytic index ( ), T and B lymphocyte proliferation levels, ear swelling degree, 50% hemolysis value (HC), and serum levels of immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-2 (IL-2), IL-4, IL-6, and tumor necrosis factor- (TNF-). Histopathological changes in spleen tissue were also examined. The results demonstrated that all SGP dose groups significantly improved immune organ indices, HC values, and B lymphocyte proliferation capacity; markedly reduced ear swelling degree; and significantly increased IgG, IgM, IL-2, IL-4, IL-6, and TNF- levels. Medium- and high-dose SGP groups (50 mg · kg ^{1} and 100 mg · kg ^{1}) also significantly enhanced T lymphocyte proliferation and increased both carbon clearance index (K) and phagocytic index ( ). Histopathological examination revealed that SGP alleviated pathological damage to the spleen in immunosuppressed mice. These findings indicate that Siraitia grosvenorii polysaccharides can significantly enhance immune function in cyclophosphamide-induced immunosuppressed mice.

Keywords: Siraitia grosvenorii; immunosuppression; mice; cyclophosphamide; polysaccharides

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Introduction

Siraitia grosvenorii, a characteristic plant of Guangxi, is a traditional Chinese medicinal herb with sweet taste and cool properties, used both as food and medicine, and revered as the “fairy fruit.”It is rich in polysaccharides, flavonoids, vitamins, proteins, and various trace elements (Li et al., 2014). Previous studies

have demonstrated its antibacterial (Liang et al., 2016), hypoglycemic (Zheng et al., 2011), and antitumor properties (Fu et al., 2016). Li et al. (2008) found that Siraitia grosvenorii polysaccharide (SGPS1) could enhance immune function in normal mice. Wang et al. (2001) investigated the immunomodulatory effects of mogrosides on cellular immune function, showing positive regulatory effects on cyclophosphamide-immunosuppressed mice. While previous research has established immunoenhancing effects in normal mice, the effects on immunocompromised animals remain unclear. Establishing an immunosuppressive animal model provides more definitive evidence for evaluating drug effects on immune function. Building upon previous studies, this research employed an intraperitoneal cyclophosphamide injection to establish an immunosuppressed mouse model to investigate the immunomodulatory effects of Siraitia grosvenorii polysaccharides, providing a theoretical basis for developing SGP as an immunoenhancing agent and fully utilizing the medicinal value of this traditional Chinese herb.

1. Materials and Methods

1.1 Experimental Animals and Materials

Specific-pathogen-free (SPF) Kunming mice of both sexes, weighing 18–22 g, were purchased from the Experimental Animal Center of Guangxi Medical University (License No. SCXK (Gui) 2014-0001). Animals were housed under controlled temperature (21–25°C) and humidity (40–65%) with free access to food and water.

Siraitia grosvenorii fruits were purchased from Xiangjun Pharmacy and authenticated by Professor Keliang Nong as the mature fruits of *Siraitia grosvenorii* (Swingle) C. Jeffrey (Cucurbitaceae). The processing method involved air-drying fresh fruits for several days followed by low-temperature drying and storage in a cool place, meeting the standards of the 2015 Chinese Pharmacopoeia. Cyclophosphamide was obtained from Saen Chemical Technology (Shanghai) Co., Ltd. (Batch No. 13061215). Levamisole hydrochloride was from Shandong Renhetang Pharmaceutical Co., Ltd. (Batch No. 150702). 2,4-Dinitrofluorobenzene (DNFB) was from Sinopharm Chemical Reagent Co., Ltd. ELISA kits for IgG, IgM, IL-2, IL-4, TNF- , and IL-6 were from Nanjing Jiancheng Bioengineering Institute. Concanavalin A (ConA), lipopolysaccharide (LPS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Shanghai Kanglang Biological Technology Co., Ltd.

1.2 Experimental Instruments

The following instruments were used: UV-6100S UV-Vis spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd.), analytical balance (Huazhi Scientific Instrument Co., Ltd.), GL-21M high-speed centrifuge (Changsha Xiangyi Centrifuge Co., Ltd.), Sanyo MCO-15AC incubator (Shanghai Fuchang Technology Co., Ltd.), AMR-100 automatic microplate reader (Hangzhou Aosheng Instru-

ment Co., Ltd.), and Waters 1525 high-performance liquid chromatography system.

1.3 Experimental Procedures

1.3.1 Preparation and Analysis of Siraitia grosvenorii Polysaccharides The polysaccharide extraction procedure was as follows: Siraitia grosvenorii fruits were pulverized and passed through a 40-mesh sieve, extracted with boiling water, concentrated under reduced pressure, precipitated with ethanol, deproteinized using the Sevage method, purified by AB-8 resin and Sephadex G-100 gel column chromatography, and lyophilized to obtain refined SGP. Infrared spectroscopy was performed using KBr pellet method with scanning range of 400–4000 cm ^{1}. Monosaccharide composition was determined by pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) followed by HPLC analysis using chromatographic conditions reported in the literature (Wang et al., 2015). Polysaccharide content was measured by the phenol-sulfuric acid method according to published protocols (Wei et al., 2018).

1.3.2 Animal Grouping and Model Establishment Mice were randomly divided into six groups: normal control, model control, SGP low-dose (25 mg · kg ^{1}), medium-dose (50 mg · kg ^{1}), high-dose (100 mg · kg ^{1}), and levamisole positive control groups. Except for the normal group, all other groups received intraperitoneal injections of freshly prepared cyclophosphamide solution (20 mg · kg ^{1}) for five consecutive days to establish the immunosuppressive model (Manepalli et al., 2013). Following successful model establishment, the levamisole group received levamisole at 20 mg · kg ^{1}, while SGP groups received the respective doses via intragastric administration. The model and normal groups received equal volumes of distilled water. All treatments were administered once daily for 14 consecutive days.

1.3.3 Determination of Immune Organ Indices Ten mice from each group were randomly selected. Twenty-four hours after the final administration, mice were euthanized by cervical dislocation. Body weight was recorded, and spleen and thymus were aseptically removed, cleaned, and weighed. Spleen index and thymus index were calculated as: organ weight (mg) / body weight (g).

1.3.4 Assessment of T and B Lymphocyte Proliferation Spleens from section 1.3.3 were aseptically ground and passed through a 200-mesh sieve to prepare single-cell suspensions, which were adjusted to a concentration of 2×10 cells/mL. In a 96-well culture plate, 100 L of splenocyte suspension was added per well in triplicate for both experimental and control wells. For T lymphocyte proliferation, 50 L of ConA (5 mg · L ^{1}) was added to experimental wells, while control wells received 50 L of RPMI-1640 medium. After incubation at 37°C with 5% CO for 44 hours, 20 L of MTT solution was added per well,

followed by an additional 4-hour incubation. The reaction was terminated and optical density (OD) was measured at 570 nm using a microplate reader. For B lymphocyte proliferation, LPS (10 mg · L ^{1}) replaced ConA, and the same procedure was followed. The stimulation index (SI) was calculated as: SI = OD of experimental well / OD of control well.

1.3.5 Determination of Carbon Clearance Index and Phagocytic Index Ten mice from each group were randomly selected. Twenty-four hours after the final administration, each mouse received 0.1 mL/10 g body weight of 10% India ink via tail vein injection. Blood samples (20 L) were collected from the orbital sinus at two different time points, each diluted in 2 mL of 0.1% Na CO solution, and absorbance (A) was measured at 680 nm. Mice were then euthanized, and immune organs were weighed to calculate the carbon clearance index (K) and phagocytic index ( ), where t and t represent the time intervals between the two blood collections.

1.3.6 Assessment of Delayed-Type Hypersensitivity Ten mice from each group were randomly selected. Twenty-four hours after the final administration, a 3 cm × 3 cm area on the abdominal wall was depilated. The following day, 50 L of 1% DNFB was applied to the depilated area. Five days later, 10 L of DNFB was applied to the right ear. After 24 hours, mice were euthanized and 6 mm diameter ear punches were taken from both ears to compare weight differences.

1.3.7 Determination of Serum Hemolysin, IgG, and IgM Levels Ten mice from each group were randomly selected. Twenty-four hours after the final administration, 0.2 mL of 5% chicken red blood cell suspension was injected intraperitoneally. Seven days later, serum was collected and diluted 100-fold with physiological saline. One milliliter of diluted serum was mixed with 0.5 mL of 5% chicken red blood cell suspension and 0.5 mL of complement, then incubated at 37°C for 30 minutes before termination in an ice-water bath. After centrifugation, the supernatant was collected and OD was measured at 540 nm. A 50% hemolysis tube was prepared by diluting 0.2 mL of 5% chicken red blood cell suspension to 2 mL with physiological saline and processed similarly to calculate the 50% hemolysis value (HC) (Gong et al., 2016; Li et al., 2012). Serum IgG and IgM levels were determined by ELISA according to the kit instructions.

1.3.8 Determination of Serum IL-2, IL-4, IL-6, and TNF- Levels Ten mice from each group were randomly selected. Twenty-four hours after the final administration, blood was collected from the orbital sinus, and serum was obtained by centrifugation. IL-2, IL-4, IL-6, and TNF- levels were measured by ELISA. Microplates were divided into blank, standard, and sample wells. Blank wells received 50 L of standard diluent, standard wells received 50 L of various standard concentrations, and sample wells received 40 L of sample diluent plus

10 L of test serum. After incubation at 37°C for 30 minutes in the dark, plates were washed five times with concentrated wash buffer and dried. Except for blank wells, 50 L of enzyme conjugate was added to each well, followed by another 30-minute incubation at 37°C in the dark. After five washes, 50 L each of chromogen A and chromogen B were added sequentially to each well, mixed gently, and incubated at 37°C in the dark for 30 minutes. The reaction was terminated, and absorbance was measured at 450 nm with blank wells as zero reference. Standard curves were plotted to calculate cytokine concentrations.

1.3.9 Histopathological Examination of Spleen Tissue Spleen samples were fixed in 10% formalin solution, embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined for pathological changes.

Statistical Analysis

Data were analyzed using SPSS 17.0 software and expressed as mean ± standard deviation ($\bar{x} \pm s$). Differences between groups were evaluated by t-test, with $P < 0.05$ considered statistically significant.

2. Results

2.1 Characterization of Siraitia grosvenorii Polysaccharides

2.1.1 Infrared Spectroscopy Analysis [Figure 1: see original paper] shows that SGP exhibited characteristic polysaccharide absorption peaks: 3440 cm ^{1} for O-H stretching vibration, 2940 cm ^{1} for C-H stretching vibration, 1600 cm ^{1} for asymmetric stretching vibration of COO from uronic acids, 1415 cm ^{1} for asymmetric stretching vibration of C=O, 1300–1000 cm ^{1} for pyran ring stretching vibration, and 765 cm ^{1} indicating the presence of D-xylose.

2.1.2 Monosaccharide Composition and Content Analysis The phenol-sulfuric acid method determined the polysaccharide content to be 98.2%. As shown in [Figure 2: see original paper], peaks 1, 3, 4, and 5 in the SGP sample were well separated and corresponded to the retention times of standard monosaccharides, indicating that SGP contains mannose, glucose, arabinose, and xylose, with glucose being the predominant component. These results differ somewhat from previous reports (Wang et al., 2015).

2.2 Effects of SGP on Immune Organ Indices in Immunosuppressed Mice

The thymus and spleen are critical immune organs essential for immune function, while phagocytes play a major defensive role through phagocytosis of foreign bacteria and viruses (Bing et al., 2013). Immune organ indices objectively reflect the functional status of immune organs in immunosuppressed mice. As shown in , SGP treatment groups and the levamisole group showed significantly increased

spleen and thymus indices compared with the model group ($P < 0.05$ or $P < 0.01$). The high-dose SGP group (100 mg · kg ^{1}) and levamisole group exhibited extremely significant differences ($P < 0.01$). Medium- and high-dose SGP groups (50 mg · kg ^{1} and 100 mg · kg ^{1}) and the levamisole group also showed significant differences compared with the normal group ($P < 0.05$). These results suggest that SGP can enhance spleen and thymus indices in immunosuppressed mice, possibly by repairing damaged cells and promoting regeneration and development of atrophied immune organs.

2.3 Effects of SGP on T and B Lymphocyte Proliferation in Immunosuppressed Mice

As shown in , medium- and high-dose SGP groups (50 mg · kg ^{1} and 100 mg · kg ^{1}) and the levamisole group demonstrated significantly enhanced T lymphocyte proliferation compared with the model group ($P < 0.05$ or $P < 0.01$). Additionally, all SGP dose groups and the levamisole group showed significant improvements in B lymphocyte proliferation capacity ($P < 0.05$ or $P < 0.01$), indicating that SGP promotes B lymphocyte proliferation in immunosuppressed mice.

2.4 Effects of SGP on Carbon Clearance and Phagocytic Indices in Immunosuppressed Mice

The carbon clearance index (K) and phagocytic index ( ) reflect phagocytic cell activity. shows that SGP treatment groups and the levamisole group exhibited increased carbon clearance and phagocytic indices compared with the model group. The medium-dose SGP group showed significant differences ($P < 0.05$), while the high-dose SGP group and levamisole group showed extremely significant differences ($P < 0.01$). Medium- and high-dose SGP groups and the levamisole group also differed significantly from the normal group ($P < 0.05$). These findings demonstrate that SGP can significantly enhance carbon clearance and phagocytic indices, increase phagocytic cell numbers, improve phagocytic activity, and strengthen innate immunity in immunosuppressed mice.

2.5 Effects of SGP on Delayed-Type Hypersensitivity in Immunosuppressed Mice

Delayed-type hypersensitivity is primarily mediated by T lymphocytes and plays an important role in cellular immunity (Venarske et al., 2003; Dale et al., 2003). Using DNFB to induce delayed-type allergic reactions, ear swelling degree serves as an indicator of cellular immune capacity. As shown in , all SGP dose groups and the levamisole group exhibited significantly reduced ear swelling compared with the model group ($P < 0.05$ or $P < 0.01$). The high-dose SGP group (100 mg · kg ^{1}) and levamisole group showed extremely significant differences ($P < 0.01$) and also differed significantly from the normal group ($P < 0.05$). These results indicate that SGP can markedly reduce ear swelling, restoring it to near-normal levels.

2.6 Effects of SGP on Serum Hemolysin, IgG, and IgM Levels in Immunosuppressed Mice

Following antigen stimulation, the body produces abundant immunoglobulins (Ig) that specifically bind antigens and enhance immune function. Serum hemolysin OD values, HC , and IgG/IgM levels reflect humoral immune strength. As shown in , SGP treatment groups and the levamisole group showed increased hemolysin OD values, HC , and IgG/IgM levels compared with the model group. High- and medium-dose SGP groups exhibited significant differences in hemolysin OD and HC values ($P < 0.05$). The low-dose SGP group (25 mg · kg ^{1}) showed significantly increased IgG and IgM levels ($P < 0.05$), while medium- and high-dose groups (50 mg · kg ^{1} and 100 mg · kg ^{1}) differed significantly from both model and normal groups ($P < 0.05$ or $P < 0.01$). These findings demonstrate that SGP can enhance antibody levels and improve humoral immune function in immunosuppressed mice.

2.7 Effects of SGP on Serum IL-2, IL-4, IL-6, and TNF- Levels in Immunosuppressed Mice

IL-2, IL-4, and IL-6 are cytokines secreted during cellular immunity that promote immune cell proliferation and differentiation, while TNF- plays crucial roles in immune defense by enhancing the cytotoxic capacity of T cells and other killer cells. As shown in , all SGP dose groups and the levamisole group exhibited elevated serum levels of IL-2, IL-4, IL-6, and TNF- compared with the model group, with significant differences ($P < 0.05$ or $P < 0.01$). Medium- and high-dose SGP groups and the levamisole group also differed significantly from the normal group ($P < 0.05$). These results indicate that SGP can promote cytokine secretion in immunosuppressed mice.

2.8 Effects of SGP on Spleen Histomorphology in Immunosuppressed Mice

[Figure 3: see original paper] reveals that normal group mice exhibited neatly arranged, structurally intact spleen tissue without inflammatory cell infiltration. The model group showed disorganized splenocyte arrangement, structural damage, inflammatory infiltration, and necrotic foci. The levamisole group displayed relatively orderly cell arrangement with consistent cell size and intact structure, though some cells showed swelling with mild cytoplasmic loosening and inflammatory infiltration, indicating therapeutic efficacy. SGP treatment groups exhibited varying degrees of improvement in splenocyte arrangement, cellular swelling, inflammatory infiltration, and necrosis. Histopathological observation confirms that SGP can effectively restore damaged splenocytes in immunosuppressed mice.

3. Discussion and Conclusion

Recent advances have revealed significant progress in understanding the immunomodulatory effects of polysaccharides from traditional Chinese medicine (Sun et al., 2015; Gong et al., 2015). Traditional Chinese medicine emphasizes enhancing disease resistance by modulating immune function. Studies have shown that plant polysaccharides can enhance immune function by repairing damaged immune cells, promoting lymphocyte cytokine secretion and activity, enhancing macrophage phagocytic activity, improving natural killer cell cytotoxicity, and accelerating immune cell proliferation and differentiation (Shang et al., 2015).

The thymus and spleen are vital immune organs where T and B lymphocytes proliferate and differentiate, making them crucial sites for immune regulation. Histopathological observations confirmed that SGP effectively restored damaged splenocytes in immunosuppressed mice. The results demonstrate that SGP significantly increased immune organ indices, possibly by repairing destroyed cells and stimulating regeneration and development of atrophied spleen and thymus tissue. Additionally, SGP enhanced T and B lymphocyte proliferation and transformation capacity, thereby improving cellular immune function.

Carbon clearance experiments showed that medium and high doses of SGP significantly increased carbon clearance index (K) and phagocytic index ( ), elevating innate immune levels in immunosuppressed mice. The mechanism may involve increasing phagocytic cell numbers, enhancing phagocytic activity, improving foreign antigen clearance, and augmenting natural killer cell cytotoxicity.

Detection of IgG, IgM, and cytokine levels helps elucidate humoral immune mechanisms at the molecular level. The results indicate that SGP increased serum hemolysin levels and significantly elevated IgG and IgM content, facilitating clearance of harmful antigens, suppressing inflammatory responses, and activating the complement system. Furthermore, SGP promoted secretion of IL-2, IL-4, IL-6, and TNF- , enhancing cytokine activity, promoting immune cell proliferation and differentiation, and strengthening cytotoxic capacity, thereby augmenting humoral immune function.

In conclusion, Siraitia grosvenorii polysaccharides can antagonize cyclophosphamide-induced immunosuppression by promoting innate, cellular, and humoral immunity, restoring disease resistance and significantly enhancing immune function in immunosuppressed mice. These findings provide a theoretical basis for developing SGP as an immunoenhancing agent.

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