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Immunomodulatory effects of ginseng glycopeptides in cyclophosphamide-induced immunosuppressed mice

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ABSTRACT

Ginseng glycopeptide complex (GGpC) refers to the mixture of polysaccharides, polypeptides and glycopeptides from ginseng. We investigated the immunomodulatory effects of GGpC and compared the effects of three GGpCs with molecular weights of 14, 11 and < 1 kDa on the immunological activity of cyclophosphamide (CTX)-induced immunosuppressed mice. These three GGpCs were obtained from the total extract of GGpC by membrane separation technology, and their immunomodulatory effects were investigated by testing carbon clearance, delayed-type hypersensitivity (DTH) response, splenic NK cell activity and counting the number of leukocytes in peripheral blood. The results showed that the total extract of GGpC and the 3-kinds of GGpC with different molecular weights increased immune organs indices, DTH response, the leukocytes number, activities of NK cells and macrophages in immunosuppressive mice. GGpC with molecular weight < 1 kDa was more effective on improving cellular immunity and NK cell activity, while GGpC with molecular weight of 14 kDa improved the function of macrophages phagocytosis. These findings indicated that GGpC had immunomodulatory effects in immunosuppressive mice, which were in inverse proportion to its molecular weights, i.e. GGpC with lower molecular weight had higher effect.

Keywords: Cyclophosphamide, different molecular weight, ginseng glycopeptide complex, immunomodulatory effect, immunosuppressed mice.

INTRODUCTION

Immunity is a physiological activity, in which the human body recognizes harmful non-self-injury to maintain its stability. The immune system plays an important role in protecting against infectious diseases and cancer in human body (21,24) Covid-19 has caused global public health and economic crisis. As of January 2021, there have been over 85 million cases and 1.8 million deaths reported, mainly among middle-aged and elderly people with weak immunity (5,28,37). This incident has made people more aware of the importance of possessing strong immunity. To meet the clinical need, it is necessary to develop novel treatments to improve the immune function of humans. Natural products, especially glycopeptides with low toxicity are source materials for development of new immune-enhancing drugs (2,3,9,14).

Effects of immune-enhancing drugs in immunosuppressed animal model is closer to the actual situation of humans with low immunity. Currently, immunosuppressive drugs to

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treat allergies, autoimmune diseases and other undesired immune responses are widely used as chemical modelling drugs, such as cyclophosphamide (CTX), hydrocortisone, dexamethasone and actinomycin, among which CTX is most widely used (19,40). CTX as an immunosuppressant intensively decreased the absolute number of T cells, circulating B cells and IgG in both humans and animals (10). It decreased the synthesis and functions of nucleic acids and proteins, interfered with the macrophage's proliferation and differentiation, killed immune cells and restrained the cellular and humoral immune response (8,12,21).

Ginseng is the dry root and rhizome of *Panax ginseng* C.A. Meyer from the Araliaceae family (22,29). The genus name *Panax* has originated from "panacea", a word meaning a cure for all diseases (30,35). Ginseng has been widely used as a herbal remedy in East Asian countries since its discovery in the mountains of Manchuria in China more than 5,000 years ago (25). It is mainly produced in Northeast China, Japan, Korea and eastern Russia and has high medicinal value and many pharmacological effects, viz., immune-enhancing, anti-ageing, anti-tumor, anti-diabetic, anti-inflammatory and anti-depressant (6,7,17,36). Its main active ingredients are saponins, polysaccharides, polypeptides, proteins, glycopeptides and volatile oils (11,13). Among them, saponins are the main components responsible for ginseng's biological activities (13). Polysaccharides, proteins and other macromolecules also play an indispensable role in the medicinal value of ginseng, especially in the immunomodulatory effects (14,15,33).

Ginseng glycopeptide complex (GGpC) is a mixture of the non-saponin macromolecular compounds (polysaccharides, polypeptides and glycopeptides) from ginseng. Some studies (23,31) have shown that GGpC has hypoglycemic, anti-inflammatory and analgesic activities, however, its effects on immunomodulatory functions are not reported. The macromolecular compound structure has an important influence on biological activity. Therefore, to further clarify the pharmacodynamic material basis of ginseng's immune-enhancing effects and to explore the potential clinical application value of GGpC, we investigated the immunomodulatory effects of GGpC and compared the effects of GGpC with different molecular weights on the immunological activity of CTX-induced immunosuppressed mice. Besides, *Lentinus edodes* mycelia polysaccharide tablets (LEMPT) as an immune-promoting agent are often used clinically to improve the immunity and treat cancer combined with chemotherapy drugs (39). The main components of LEMPT are high molecular polymers of glycopeptide that are isolated from the fruiting body of *Lentinus edodes* and similar to ginseng glycopeptide (41). Therefore, it was selected as the positive control drug in this paper.

MATERIALS AND METHODS

(i). Ginseng glycopeptide complex (GGpC) of different molecular weights

The 5-year-old *Panax ginseng* (sun-dried ginseng) plants were bought from Jilin Yisheng Foreign Trade Co., Ltd. (Changchun, China) and authenticated by Professor Chen Changbao in Jilin Ginseng Academy, Changchun University of Chinese Medicine, China. Five hundred g dried ginsengs roots were pulverized and decocted thrice with distilled

water (7 L), 1 h each time. The extract was concentrated and mixed with 70% ethanol, and then incubated at 4 ℃ for 24 h, finally filtered to obtain the precipitate. The precipitate (116.5 g) was freeze-dried and hydrolysed with flavourzyme (Henan Wanbang Industrial Co., Ltd., Henan, China) and papain (Zhejiang Yinuo Biological Technology Co., Ltd. Zhejiang, China) in the ratio of 1:1 (w/w). The parameters of the mixed enzymolysis were: 1.0 % enzyme, initial pH 6.0, temperature 50 °C, and hydrolysis time 4 h. The hydrolysate was inactivated in boiling water bath for 10 min, cooled to room temperature, centrifuged at 4000 rpm (rounds per minute) for 15 min, and then the supernatant was freeze-dried to obtain the ginseng glycopeptide complex (GGpC, 85.1 g).

The GGpC (70.0 g) was dissolved in water, centrifuged to remove the precipitate (27.2 g) and the supernatant was dialyzed for 24 h through 8000-14000, 7000, 3500, 1000, 500 Da dialysis membranes successively. The internal and external dialysate were collected, concentrated and freeze-dried and their molecular weights were determined by high-performance liquid chromatography (HPLC). Based on molecular weights, ginseng glycopeptides were divided in 3-groups: (i). 14 kDa (GGpC-1, 22.6 g, 85.1 %), (ii). 11 kDa (GGpC-2, 8.0 g, 89.8 %) and (iii). < 1 kDa (GGpC-3, 7.2 g, 86.4 %).

(ii). Molecular weight distribution

The molecular weight distributions of 3-kinds of ginseng glycopeptides were determined by HPLC (LC-20A, Shimadzu, Japan) on the SRT SEC-100 column (7.8 × 300 mm, 5 μm; Sepax Technologies, America). The data were obtained using the following conditions: column temperature 35 ºC, mobile phase 0.7 % sodium sulfate, flow rate 0.5 mL/min, injection volume 20 μL. Dextran (Sigma-Aldrich) with molecular weights of 180-12000 Da were used as the standards and the results were analyzed by GPC software.

(iii). Experimental animals and protocols

BALB/c mice (female, 6-8 weeks old, 20 ± 2 *g* body weight) were purchased from the Changsheng Biotechnology Co., Ltd. (Liaoning, China). The mice were kept in a standard lab environment with pathogen-free conditions (24-25 ºC, 45-55 % humidity, 12 h light-dark cycle). All animal experimental protocols were followed in accordance with local guidelines for the care of laboratory animals of Changchun University of Chinese Medicine, and were approved by the Ethics Committee of Animal Care and Welfare for research on laboratory animal use of the institution [SCXK (Ji) 2018-0013].

Two hundred and seventy mice were randomly assigned into 3-batches with 9-groups per batch and 10-mice per group. The dosages of GGpC-1, GGpC-2, and GGpC-3 groups were equivalent to that of GGpC-H, which were converted according to their extraction rate in ginseng, respectively. After one-week adaptation, the Normal control (NC, being feeded normally without treatment) and Model control (MC, an animal model of immunosuppression induced by cyclophosphamide, without treatment) groups were applied with 10 mL/kg deionized water, while the other groups (GGpCs treatments in immunosuppressed animals) were intragastrically administered with the corresponding test solution in the same way once a day for 30 days. During the intragastric administration, from day 22, 50 mg/kg cyclophosphamide (CTX, Baxter Oncology GmbH, Halle, Germany) was injected intraperitoneally 5 times every two days for Positive control [PC,

treated with *Lentinus edodes* mycelia polysaccharide tablets (LEMPT, Kaifeng Pharmaceuticals, Henan, China)], MC, GGpC-L, GGpC-M, GGpC-H, GGpC-1, GGpC-2, and GGpC-3 groups (Table 1) (6). The treatments details are given in Table 1.

Table 1. Experimental treatments and their doses used in animal experiments.

No.	Treatments	Intragastric administration	Intragastric administration dose	CTX dose
1	Normal control (NC)	Deionized water	10 mL/kg	-
2	Model control (MC)	Deionized water	10 mL/kg	50 mg/kg/d
3	Positive control (PC)	LEMPT	0.67 mg/kg/d	50 mg/kg/d
4	Low dose of GGpC (GGpC-L)	GGpC	70 mg/kg/d	50 mg/kg/d
5	Medium dose of GGpC (GGpC-M)	GGpC	140 mg/kg/d	50 mg/kg/d
6	High dose of GGpC (GGpC-H)	GGpC	210 mg/kg/d	50 mg/kg/d
7	GGpC with molecular weight of 14 kDa (GGpC-1)	GGpC-1	93.4 mg/kg/d	50 mg/kg/d
8	GGpC with molecular weight of 11 kDa (GGpC-2)	GGpC-2	33.6 mg/kg/d	50 mg/kg/d
9	GGpC with molecular weight less than 1 kDa (GGpC-3)	GGpC-3	29.4 mg/kg/d	50 mg/kg/d

(iv). Assay of spleen and thymus indices

After 30 days of experiment, the mice were weighed and then killed by cervical dislocation. The spleens and thymuses were quickly separated and weighed to calculate the spleen and thymus indices according to the equation below (34) :

$$\text{Spleen or thymus index} = \text{spleen or thymus weight (mg)/body weight (g)}$$

(v). Assay of carbon clearance test

The phagocytic activity of macrophages was assessed by the carbon clearance method. Briefly, 0.1 mL/10g Indian ink (Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China) which was diluted 4 times with sterile saline was administered intravenously into the caudal vein to all 9-groups of mice on day 31. Blood samples (20 μL) were collected from the ophthalmic venous plexus at 2 and 10 min (named as t_1 and t_2) after the injection. 2 mL of 0.1 % sodium carbonate solution (Shanghai Yuanye Biotechnology Co., Ltd., Shanghai, China) was added to the samples. The absorbance values of samples were measured at 600 nm using a multifunction microplate reader (M200 pro-type, TECAN Group Company, Switzerland), and sodium carbonate solution was used as a blank control in the measurement. After collecting blood, the mice were killed by cervical dislocation, and the spleens and livers were separated and weighed. The phagocytic index α was used to validate the function of mice phagocytes in clearing carbon particles, and it was calculated by the following equation (34):

$$\alpha = \text{body weight} \times \sqrt[3]{K} / (\text{liver weight} + \text{spleen weight}) \; ; \quad K = (\lg OD_1 - \lg OD_2)/(t_2 - t_1)$$

Where, OD_1 and OD_2 are the optical densities at t_2 (10 min) and t_1 (2 min) respectively, and the slope K is the phagocytosis rate.

(vi). DNFB-induced delayed type hypersensitivity (DTH) response

On day 25, the abdominal skins of all the animal groups were depilated with depilatory cream (Veet, Reckitt Benckiser Plc. (China) Co., Ltd., China) of about 3 cm × 3 cm, and then sensitized by 50 μL dinitrofluorobenzene (DNFB, Macklin Biochemical Technology Co. Ltd., Shanghai, China) solution smeared on the shaved abdomen evenly. Five days after skin allergy, the right ears of all mice were smeared by 10 μL of DNFB solution, while the left ears were used as control. After 24 h, the left and right ears were cut off after the mice were killed by cervical dislocation. The round ear pieces (6 mm diameter) were taken from each earlap using a perforator and then weighed. The degree of ear swelling, that is, the difference in the weight between the right and left ear piece, was used as the evaluation index of DTH response (18).

(vii). Leukocyte count in peripheral blood

The whole blood samples collected from retro-orbital plexus of mice were mixed well to prevent blood clotting and then detected the leukocyte numbers by a whole blood cell analyzer (Sysmex, XT200i, Japan) within 24 h (27).

(viii). Splenic NK cell activity

The splenic natural killer (NK) cell activity was determined by the lactate dehydrogenase (LDH) releasing method. The spleen was taken from the mice killed under aseptic conditions, washed with sterile Hank's solution (Gibco, Grand Island, NY, USA), and gently crushed with forceps to obtain a uniform cell suspension. The obtained cell suspension was washed twice with Hank's solution and centrifuged at 1000 rpm for 10 min. The supernatant was discarded to reclaim the splenocytes. The reclaimed splenocytes were resuspended in red blood cell lysing buffer (Sigma, St. Louis, MO, USA) for 5 min to remove red blood cells. After centrifugation at 1000 rpm for 10 min, the splenocytes were re-suspended in RPMI1640 complete medium containing 10 % fetal bovine serum (Gibco, Grand Island, NY, USA) and then adjusted the cell concentration to 2×10^7 cells/mL that were taken as effector cells.

The 100 μL targe YAC-1 cells (4×10^5 cells/mL, Cell Bank of Chinese Academy of Sciences, Shanghai, China) was added to the well which contained the 100 μL effector cells to ensure a 50:1 effector/target ratio. The spontaneous release level and the maximum release level of each well were examined. 100 μL of target cells and 100 μL of culture medium were added to the spontaneous releasing well, while 100 μL of target cells and 100 μL of 2.5 % Triton (Solarbio, Beijing, China) were added to the maximum releasing well. All tests in wells were done thrice. After incubation for 4 h in a 37 ℃, 5 % CO_2 incubator (Thermo Fisher Scientific, LS-CO150, USA), the cells on each plate were centrifuged at 1500 rpm for 5 min. Subsequently, 100 μL of supernatant was removed to another 96-well plate mixture containing 100 μL LDH substrate solution. After reaction for 3 min, 30 μL of 1 mol/L HCl solution was applied to each well. The absorbance values were measured at 490 nm using a multifunction microplate reader and the splenic NK cell activity was calculated using the following equation (27,38):

$$\text{NK cell activity (\%)} = \frac{\text{OD reaction-OD spontaneous}}{\text{OD maximum-OD spontaneous}} \times 100\%$$

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed by one-way analysis of variance (ANOVA), and the data were statistically analyzed using t-test. Multiple comparison and graph plotting was performed by GraphPad Prism 7.0 software package (GraphPad Inc., San Diego, USA). P-value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Molecular weight distribution

The average molecular weight and molecular weight distribution of ginseng glycopeptides were analyzed by HPLC and GPC software for further investigation of the effects of ginseng glycopeptides with different molecular weight on the immune activity of mice. Three kinds of ginseng glycopeptides with different molecular weights were isolated from dialysis membranes (Table 2).

Table 2. Molecular weight distribution in ginseng glycopeptide complex (GGpC).

Groups	Average of molecular weight (kDa)	Proportions of molecular weights (%)				
		> 14~8 kDa	14~8-7 kDa	7-1 kDa	1-0.5 kDa	< 0.5 kDa
GGpC-1	13.8670 (14)	25.2301	58.8455	3.8765	0.8220	11.2258
GGpC-2	11.0860 (11)	19.5698	41.3038	2.4822	3.2714	33.3719
GGpC-3	0.4460 (< 1)	—	—	—	10.0240	89.976

Effects of GGpC on the spleen and thymus indices

Low, medium, high doses of GGpC, high doses of GGpC-1, GGpC-2, and GGpC-3 were orally administered to CTX-induced immunosuppressed mice for 30 consecutive days, to investigate the immune regulatory effects of GGpC and compared the immune activity of ginseng glycopeptide with different molecular weights. As shown in Table 3, the body weight of mice in the GGpC groups was similar to normal control group (NC), indicating that ginseng glycopeptides had no toxic effects on mice.

The thymus and spleen are two major immune organs accountable for initiating immune reactions in the body (6). The relative weights of the thymus and spleen are recognized as critical and intuitive indices for non-specific immunity, and they will shrink when the immune function declines (21). In our study, the thymus and spleen indices in the MC group were significantly decreased compared to NC (P < 0.01). However, all the GGpCs treatment groups showed a dramatic increase in the thymus and spleen indices compared to the MC group (P < 0.05, P < 0.01). Moreover, the spleen index in the GGpC-2 group was higher than GGpC-1 and GGpC-3 groups (Table 3). This result showed that GGpC had a protective effect on the reduction of spleen and thymus indices, indicating GGpC could reverse the CTX-induced atrophy of immune organs.

Table 3. Effects of ginseng glycopeptide complex (GGpC) on the spleen and thymus indices.

Groups	Body weight before treatment (g)	Body weight after treatment (g)	Spleen index (mg/g)	Thymus index (mg/g)
NC	20.51 ± 0.86	22.23 ± 0.90	4.52 ± 0.23	1.74 ± 0.14
MC	19.51 ± 0.60	21.15 ± 0.37$^{\#}$	1.72 ± 0.14$^{\#\#}$	0.30 ± 0.02$^{\#\#}$
PC	19.50 ± 1.07	22.24 ± 0.71*	2.14 ± 0.18**	0.35 ± 0.01**
GGpC-L	18.93 ± 0.62	20.89 ± 0.65	2.09 ± 0.19**	0.45 ± 0.02**
GGpC-M	19.44 ± 1.01	21.53 ± 0.90	2.45 ± 0.10**	0.48 ± 0.03**
GGpC-H	19.41 ± 0.94	22.26 ± 0.94*	2.57 ± 0.05**	0.49 ± 0.01**
GGpC-1	19.21 ± 0.83	21.14 ± 0.90	2.18 ± 0.41*	0.48 ± 0.02**
GGpC-2	19.25 ± 0.77	21.15 ± 0.72	2.29 ± 0.21**	0.47 ± 0.02**
GGpC-3	19.03 ± 0.69	22.98 ± 0.68*	1.88 ± 0.04*	0.46 ± 0.03**

Note: Results are given as Mean ± SD (n = 10) in each group, and a one-way ANOVA procedure followed by the t-test was used to evaluate the statistical significance. Significance at $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ when compared to normal group; $*P < 0.05$, $**P < 0.01$ when compared to model control group. NC: normal control group; MC: model control group, treated with CTX (50 mg/kg); PC: positive control group, treated with LEMPT (0.67 mg/kg/d); GGpC groups: treated with GGpC at dosages of 70 mg/kg/d (L), 140 mg/kg/d (M) and 210 mg/kg/d (H), and also treated with GGpCs at molecular weight of 14 kDa (1), 11 kDa (2) and < 1 kDa (3).

Effects of GGpC on macrophages activity

Macrophages play a crucial role in host defense mechanisms in which many immunomodulatory factors activate the immune response depending on the activation of macrophages (21). Macrophage phagocytosis, as a marker to measure non-specific immunity, is one of the important indicators to evaluate the immune functions (26).

The phagocytic activity of macrophages was assessed by the carbon clearance method. As per Figure 1, the phagocytic activity of macrophages in the CTX-treated group (MC group) was significantly reduced ($P < 0.05$), compared with the NC group. All GGpC treated groups, especially the medium, high doses of GGpC and 14 kD GGpC treated groups, had an extremely significant increase in the phagocytosis indices of macrophages, compared with the MC group ($P < 0.01$). Similarly, the phagocytosis indices of macrophages in the low dose, 11 kD and <1 kD of GGpC treated groups were also significantly increased ($P < 0.05$). The phagocytosis index of macrophages in the GGpC-1 group was similar to that of GGpC-H group ($P > 0.05$), while the phagocytosis index of macrophages in the GGpC-2 and GGpC-3 groups was significantly decreased compared with that of the GGpC-H group ($P < 0.05$). These results showed that GGpC increased phagocytic index in CTX-treated mice, which is an indicator of the elevated non-specific immune response. Moreover, the phagocytic ability of GGpC was related to the dose and molecular weight of GGpC. GGpC with a high dose and high molecular weight improved the functioning of macrophages phagocytosis.

Effects of GGpC on DTH

DTH is an important type of cell-mediated pathologic response and plays a key role in the evaluation of T-cell mediated immune responses (21,32). DTH requires the specific recognition of a given antigen by the activated T lymphocytes, which subsequently proliferates and releases cytokines (4). In this study, we used DNFB as the antigen-induced DTH reaction on mice ears to evaluate *in-vivo* effects of GGpC on cellular immune response.

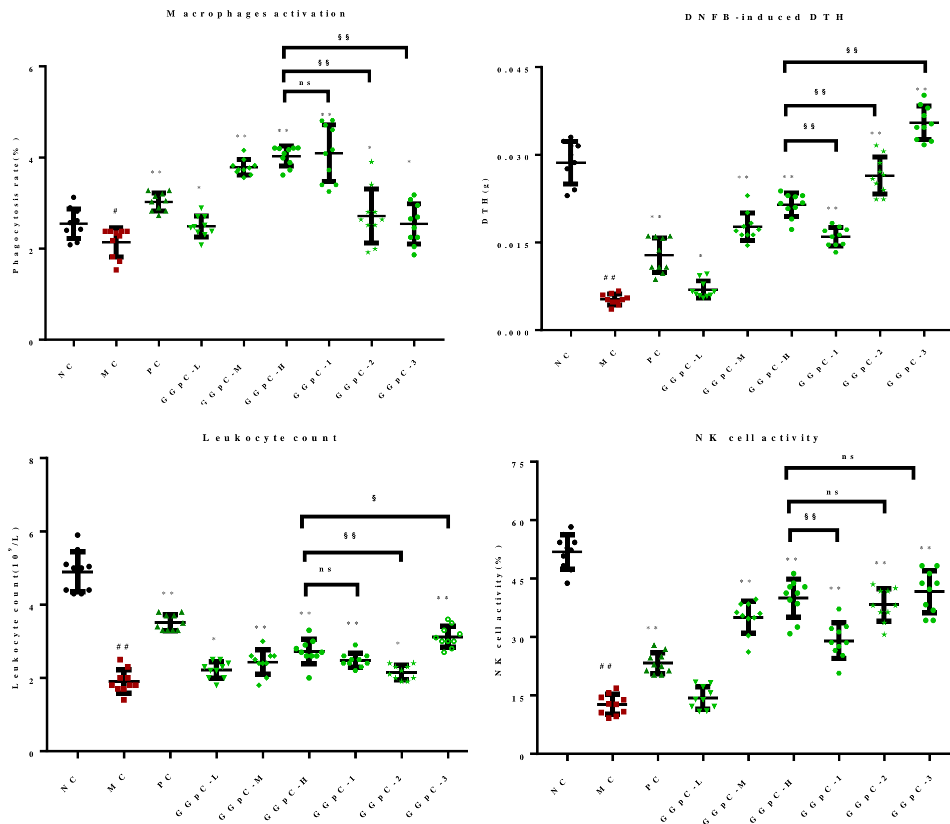

Figure 1. Effects of ginseng glycopeptide complex (GGpC) on macrophages activation, DNFB-induced DTH, leukocyte count and NK cell activity. Results are given as Mean ± SD (n = 10) in each group. Significance at $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ when compared to normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to model group; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, ns $P > 0.05$ when compared to GGpC-H group.

The results of DTH response showed a dose-related increase in the degree of ear swelling due to treatment with three doses of GGpC ($P < 0.05$ or $P < 0.01$; Figure 1). Similarly, there was an increase in ear swelling degree in groups treated with three GGpCs of different molecular weights ($P < 0.01$) compared with the MC group. The degree of ear swelling in the GGpC-1 group was significantly lower than that in GGpC-H group ($P < 0.01$), while the degrees of ear swelling in the GGpC-2 and GGpC-3 groups were markedly higher than that in the GGpC-H group ($P < 0.01$). It showed that the GGpC counteracts the inhibitory effect of CTX on the DTH reaction and enhanced the cell-mediated immune function in CTX-treated mice. The results also suggested that lower molecular weight of GGpC is more effective on cellular immunity enhancement.

Effects of GGpC on leukocyte count

Leukocytes are blood cells involved in the immune response and the leukocyte number is an important clinical parameter in the diagnosis and prognosis of various diseases (20). The leukocyte number of the MC group was significantly decreased than that of NC group ($P < 0.01$; Figure 1). Compared with the MC group, the leukocyte number of GGpC groups was significantly increased ($P < 0.05$ or $P < 0.01$). There was remarkably higher leukocyte number in the GGpC-3 group than that in GGpC-H group ($P < 0.05$), while the leukocyte number was significantly decreased in the GGpC-2 group compared with GGpC-H group ($P < 0.01$). These showed that GGpC could promote the formation of leukocytes in CTX-immunosuppressed mice and there was no influence of molecular weight on the leukocyte number.

Effects of GGpC on NK cell activity

NK cells are important components in the innate immune system and play a crucial role in the anti-viral infection, in which NK cells kill the target cells of tumor cells and viruses, without prior sensitization by antigen and involvement of specific antigen or complement (1,16). Determination of NK cell activity is used to evaluate the immune responses of infections. In our study, the LDH method was used to determine the NK cell activity in CTX-induced immunosuppressed mice. The NK cell activity in the MC group was significantly decreased than that in the NC group ($P < 0.01$; Figure 1). However, compared with the MC group, NK cell activity in different doses of GGpC treated groups (GGpC-M, GGpC-H) was significantly increased in a dose-dependent manner ($P < 0.01$). Similarly, treatments with different molecular weights of GGpC significantly increased NK cell activity compared with MC ($P < 0.01$). There was no significant difference in NK cell activity between GGpC-2, GGpC-3, and GGpC-H groups ($P > 0.05$), while it was markedly lower in GGpC-1 group than in GGpC-H group ($P < 0.01$). Moreover, the NK cell activity in groups treated with medium, high doses and different molecular weights of GGpC were comparable to LEMPT (PC group). Results indicated that the GGpC increased the activity of NK cells in CTX-treated mice. Thus, GGpC might modulate immune responses through promoting NK cell activity in CTX-treated mice *in-vivo*.

CONCLUSIONS

The GGpC successfully increased the immune organ indices and NK cell activity, and improved cellular immunity and monocyte-macrophage function in CTX-induced immunosuppressed mice. Moreover, the immunization of GGpC with lower molecular weight was relatively better than that of GGpC with higher molecular weight. These findings provided evidences of the immuno-enhancing effects of GGpC, and suggested that GGpC had potential applications in the treatment of immunosuppressive diseases. These results will provide guidance for further separation and purification of GGpC. Further studies on GGpC are needed to understand its action mechanism of enhancing immune function.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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