Comparison of different effective parts of Panax Ginseng in enhancing immune function of mice

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Research Article

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Abstract

The present study focused on comparing the effects of different active parts of five-year-old ginseng on the immunological activity of cyclo-phosphamide (CTX)-induced immunosuppressed mice. Preparation of ginseng water extract, total ginsenoside complex, ginseng glycoprotein complex, ginseng glycopeptide complex, by water extraction and alcohol precipitation method and enzymolysis method. The immunomodulatory effects of different effective parts of ginseng were determined by carbon clearance test, immune organ index, delayed type hypersensitivity (DTH) response, leukocyte count in peripheral blood, spleen lymphocyte transformation experiment, splenic NK cell activity, serum hemolysin and Experiment on the determination of cytokines. The results showed that ginseng water extract, total ginsenoside complex, ginseng glycoprotein complex and ginseng glycopeptide complex improved immune organs indices, DTH response, leukocyte count, NK cell activity, cytokine content, hemolysin content and the phagocytosis of macrophages in immunosuppressive mice. Ginseng glycoprotein complex and ginseng glycopeptide complex had more effectiveness on cellular immunity. Total ginsenoside complex and ginseng glycoprotein complex can better enhance the humoral immune function of mice. Total ginsenoside complex, ginseng glycoprotein complex and ginseng glycopeptide complex had a better effect on improving the function of macrophages phagocytosis. The enhancing effect of ginseng glycoprotein on NK cell activity was better than other extracts. Total ginsenoside complex, ginseng glycoprotein complex and ginseng glycopeptide complex can significantly increase the content of TNF-*α* in mice, ginseng water extract can significantly in-crease the content of IL-2, ginseng water extract and total ginsenoside complex are more effective in enhancing IL-6 levels in mice. Different effective parts of ginseng can improve the immunity of mice, and the effect of ginseng glycoprotein is more obvious. It is speculated that ginseng glycoprotein is the main active site of ginseng against CTX immunosuppression. This study laid a theoretical foundation for further study on the pharmacodynamic material basis and mechanism of ginseng to improve immunity.

Introduction

An active part is an extract obtained from a single plant, animal, mineral, etc., while Chinese medicine contains many different active parts with multiple components and multiple targets. Therefore, the material basis of the medicinal effect of traditional Chinese medicine is not one functional part, but a joint action of different effective parts, and at the same time there is a single effective part showing a multi-target phenomenon (Chen et al. 2020, Jin et al. 2019, Wang et al. 2020). For example, Li et al. compared three different antioxidant detection methods of Cordyceps. The results showed that different components of Cordyceps Sinensis may affect different forms of antioxidant effects (Li et al. 2001). Nowak et al. observed the effect of different extraction processes on the antioxidant capacity of Silybum marianum L. and found that there were significant differences in the antioxidant capacity of the extracts from different extraction methods (Nowak et al. 2021). These studies have shown that traditional Chinese medicine, as a complex system, may have different components that play a major therapeutic

role in a certain disease, and may also have different functional activities for a certain component (Zeng et al. 2019).

Panax ginseng C. A. Mey., as a traditional and valuable Chinese herbal medicine, has been used to treat medical diseases or maintain homeostasis due to its safety and multi-target advantages (Guo et al. 2021, Kang and Min 2012, Wu et al. 2018). In recent years, with ginseng being classified as a new resource food, its edible value is trusted and loved by people (Patel and Rauf 2017), and people are now more concerned about the research and development of new resource functional food with the main raw material of herbal medicine of the same source, aiming to improve people's health and enhance their health level (Zhang et al. 2021). With the development of modern separation and analysis technology and the progress of medical science, it was found that *Panax ginseng* C. A. Mey. contained ginsenosides, glycoproteins, glycopeptides, polysaccharides, polypeptides, and other components, and had good effects on improving immunity. Nowadays, *Panax ginseng* C. A. Mey. is considered to be an immunomodulator, which can play an immunoregulatory role in various ways, such as protecting the thymus and spleen of the inherent immune organs and maintaining the immune system of the body; improving the immune response level of inherent immune cells such as macrophages, NK cells, and killing virally infected cells and tumor cells; regulate the secretion of immune molecules such as cytokines and complements to exert immune enhancement. As reported by the relevant literature, low immunity will cause many diseases, therefore, *Panax ginseng* C. A. Mey. immune regulation and its mechanism of action have been widely concerned (Chen et al. 2019, Choi et al. 2014, Luo et al. 2018, Quan et al. 2007, Shan et al. 2021, Yang et al. 2017, Zhang et al. 2018). The immunomodulatory effects of *Panax ginseng* C. A. Mey. may originate from the various important bioactive substances contained in *Panax ginseng* C. A. Mey., while some studies in recent years have found that ginsenosides, ginseng polysaccharides, ginseng proteins, and ginseng oligopeptides in *Panax ginseng* C. A. Mey. have immunomodulatory effects, and mainly focus on ginsenosides and ginseng polysaccharide components as research objects (Hu et al. 2020, Li et al. 2019, Wang et al. 2018, Fan et al. 2020), but there is less research on the components in *Panax ginseng* C. A. Mey. that mainly have immune-enhancing effects. Therefore, this study intends to prepare different effective parts of *Panax ginseng* C. A. Mey. by water extraction and alcohol precipitation, and enzymatic hydrolysis. The immunoregulatory activities of different effective parts are studied and compared by establishing a cyclophosphamide-induced immunosuppression model, aiming to explore the effective substances that can truly improve the immunity of *Panax ginseng* C. A. Mey. and expand its potential clinical value.

Materials And Methods

Reagents and Materials

Food grade flavored protease, purchased from Henan Wanbang Industrial Co. (Henan, China). Food grade papain, purchased from Zhejiang Yino Biotechnology Co. (Zhejiang, China). BCA Protein Concentration Assay Kit (Enhanced) purchased from Shanghai Biyuntian Biotechnology Co. (Shanghai, China). Cyclophosphamide injection, size 200 mg/stem, purchased from Baxter Oncology GmbH (Halle,

Germany). Lentinus Edodes Mycelia Polysaccharide Tablets, size 15 mg/tablet, purchased from Kaifeng Pharmaceutical (Group) Co. (Henan, China), lot number: 20010104. DNFB was purchased from Shanghai Maclean Biochemical Technology Co. (Shanghai, China). RPMI-1640 medium was purchased from Gibco (Grand Island, NY, USA). 10% calf serum purchased from Tianjin Kang Yuan Biotechnology Co. (Tianjin, China), item no. KY-01003. YAC-1 was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Dipotassium EDTA, India ink, and Na_2CO_3 were purchased from Shanghai Yuanye Biotechnology Co. (Shanghai, China). Penicillin and streptomycin double antibodies, Cuttlefish protein A (ConA), MTT, PBS buffer (pH 7.2-7.4), SRBC, Hank's solution, nitro tetrazolium chloride, phenazine dimethyl sulfate, oxidized coenzyme I, 0.2 mol/L Tris-HCl buffer, 2.5% Triton all purchased from Solarbio (Beijing, China). Cytokines, including IL-2, IL-6, and TNF-*α*, were provided by Invitrogen Co., Ltd. (Carlsbad, CA, USA). All other reagents are of analytical purity grade.

Preparation of Different Effective Parts of Ginseng

The 5-year-old *Panax ginseng* (sun-dried ginsengs) were bought from Jilin Yisheng Foreign Trade Co., Ltd. (Changchun, China) and authenticated by Professor Changbao Chen in Jilin Ginseng Academy, Changchun University of Chinese Medicine, China. The ginseng was pulverized (60 mesh) and then decocted with water (7 L, 3 times) for 1 h. The extract was concentrated under reduced pressure to obtain the water extract (GWE). Part of the water extract was precipitated with 70% ethanol and stored at 4 ℃ for 24 h. After filtration, the supernatant was freeze-dried to obtain the crude extract of ginseng total saponins extract (TGC), and the precipitate was freeze-dried to obtain the crude extract of ginseng glycoprotein complex (GGC-1). Ginseng glycoprotein was hydrolyzed with flavored protease and papain at (1:1, w/w). The parameters of the mixed enzymolysis were as follows: enzyme addition amount of 1.0%, initial pH 6.0, temperature 50 ℃, and hydrolysis time of 4 h. The hydrolysate was inactivated in a boiling water bath for 10 min, cooled to room temperature, centrifuged at 4000 rpm (revolutions per minute) for 15 min, and then the supernatant was freeze-dried to obtain the ginseng glycopeptide complex (GGC-2).

Animals and Experimental Design

BALB/c mice (female, 6-8 weeks old, 20 ± 2 g body weight) were purchased from Changsheng Biotechnology Co., Ltd. (Liaoning, China). The mice were kept in a standard lab environment with pathogen-free conditions (24-25 ℃, 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 at Changchun University of Chinese Medicine and were approved by the Ethics Committee of Animal Care and Welfare for research on laboratory animal use at the institution [SCXK (Ji) 2018-0013], Ethics number is 2021068.

Two hundred and eighty mice were randomly assigned into 4-batches with 7-groups per batch and 10-mice per group (Table 1). which were converted according to their extraction rate in ginseng, respectively. After one-week adaptation, the NC (Normal control, Feed normally without treatment) and MC (Model

control, An animal model of immunosuppression induced by cyclophosphamide, without drug intervention) groups were applied 10 mL/kg of deionized water, while the other groups (Drug treatment in immunosuppression animals) were treated daily 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) was injected intraperitoneally 5 times every two days for MC, PC (Positive control, Treatment with the positive drug of Lentinus edodes mycelia polysaccharide tablets (LEMPT), GWE, TGC, GGC-1 and GGC-2 groups (Table 1). These treatment details are given in Table 1.

Table 1

Experimental treatment 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	20 mg/kg/d	50 mg/kg/d
4	Ginseng water extract (GWE)	GWE	900 mg/kg/d	50 mg/kg/d
5	Total ginsenoside complex (TGC)	TGC	700 mg/kg/d	50 mg/kg/d
6	Ginseng glycoprotein complex (GGC-1)	GGC-1	300 mg/kg/d	50 mg/kg/d
7	Ginseng glycopeptide complex (GGC-2)	GGC-2	210 mg/kg/d	50 mg/kg/d

Determination of Spleen and Thymus Index

After 30 days of the experiment, the mice were weighed and sacrificed by cervical dislocation. The spleen and thymus were quickly separated and weighed, and the spleen and thymus indexes were calculated according to the following formula (Xing et al. 2017):

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

Non-specific Immune Function Experiment

Carbon Clearance Experiment. On the 31 st day, the mice were injected intravenously with India ink diluted 4 times with sterile saline, and the amount of each mouse was 0.1 mL/10 g. After injection, the time was recorded immediately. The blood (20 μL) was collected from the epicanthus venous plexus at 2 min and

10 min, respectively. The collected blood samples were immediately added with 2 mL Na_2CO_3 solution and mixed. With Na_2CO_3 solution as blank control, 200 μL of each sample was accurately extracted, and the absorbance was measured at 600 nm wavelength of the enzyme-labeled instrument. After collecting blood samples, the mice were sacrificed by cervical dislocation, and the spleen and liver were separated and weighed. The phagocytic index *α* was used to indicate the function of phagocytes in mice to remove carbon particles, and calculated by the following formula (Xing et al. 2017):

$$\alpha = \text{body weight} \times \sqrt[3]{k} / (\text{liver weight} + \text{spleen weight});$$

$$k = (\lg \text{OD1} - \lg \text{OD2}) / (t2 - t1)$$

Where, OD1 and OD2 are the optical densities at t2 (10 min) and t1 (2 min) respectively, and the slope K is the phagocytosis rate.

NK Cell Activity Assay. In this study, the activity of NK cells was determined by the lactate dehydrogenase method (Yang et al. 2009). The spleens of mice killed under sterile conditions were rinsed with sterile Hank's solution and gently crushed with clamps to obtain uniform cell suspension. The obtained cell suspension was washed twice with Hank's solution, centrifuged at 1000 rpm for 10 min, and the supernatant was removed to recover spleen cells. The recovered spleen cells were added with red blood cell lysate to remove red blood cells and then added with Hank's solution. After centrifugation at 1000 rpm for 10 min, 1 mL RPMI-1640 complete medium containing 10% calf serum was used for suspension, and the cell concentration was adjusted to 2×10^7 cells/ mL.

The 100 μL targe YAC-1 cells (4×10^5 cells/mL) were 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. Quantities of 100 μL of target cells and 100 μL of culture medium were added to the spontaneous releasing well; Quantities of 100 μL of target cells and 100 μL of 2.5% Triton were added to the maximum releasing well. All tests in wells were done thrice. After incubation for 4 h in a 37 °C, 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 sucked from each well and added to another 96-well plate mixture with 100 μL of LDH substrate solution. After reaction for 3 min, 30 μL of 1 mol/L HCl solution was applied to each well. The absorbance was measured at 490 nm using a multifunction microplate reader and the splenic NK cell activity was calculated using the following equation:

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

Specific Immune Function Experiment

Cellular Immunity Experiment: Spleen Cell Proliferation Test. The proliferation rate of cells was detected by the ConA-induced mouse spleen lymphocyte transformation experiment (Yang et al. 2009). Splenocytes were processed in the same way as for the NK cell activity assay.

The cell suspension was seeded in a 24-well culture plate, 1 mL per well, added with ConA solution 75 μL /well, and set a blank control. The cells were cultured at 37 °C for 72 h in a 5% CO_2 incubator. Four hours before the end of the culture, the culture medium of each hole was discarded, the RPM1-1640 culture medium without calf serum was added to each hole, and the concentration of 5 mg/mL MTT 50 μL was continued to culture for 4 h. After the end of the culture, gently suction the culture medium in the hole, adding I mL DMSO to each hole, blowing and mixing so that the purple crystal completely dissolved. Packed into a 96-well culture plate, each hole was packed into 6 holes as parallel samples, and the optical density was measured by the enzyme-labeled instrument at 570 nm wavelength. Zeroing with cell-free blank hole, and the proliferative capacity of splenocytes was calculated using the following equation:

$$\text{Spleen cell proliferative capacity} = \text{ODwith ConA-ODwithout ConA}$$

Cellular Immunity Experiment: Delayed-Type Hypersensitivity Reaction (DTH). At 25 d, the abdominal skin of mice in each group was depilated with depilating ointment, and 50 μL DNFB solution was evenly smeared on the depilated abdomen to sensitize it. After 5 days of skin allergy, the mice in each group were attacked with 10 μL DNFB solution on both sides of the right ear, and the left ear was used as the control. After 24 h, the mice were sacrificed by cervical dislocation, and the left and right ears were taken. The round ear lobes with a diameter of 6 mm were taken out from each ear lobe with a perforator and weighed. The degree of ear swelling (i. e., the weight difference between the left and right ear lobes) was used as the evaluation index of DTH reaction (Gaspari et al. 2016).

Humoral Immunity Experiment: Determination of Serum Hemolysin. In this study, the hemagglutination method was used to detect the level of hemolysin. 4% (v/v) of SRBC was diluted into 2% cell suspension with sterile saline at 26 d, and mice in each group were immunized by intraperitoneal injection of 0.2 mL. 4 d later, blood was removed from the eyes of mice and placed in 1.5 ml centrifuge tubes at room temperature for about 1 h. The clotted blood was stripped from the wall of the tube so that the serum was fully precipitated, centrifuged at 2000 r/min for 10 min, and the serum was collected. The serum was diluted 1, 2, 4, and 8 times with physiological saline. The serum with different dilutions was placed in a micro-hemagglutination test plate, 100 μL per well, and then 100 μL 0.5% (volume fraction) SRBC suspension was added. The suspension was mixed evenly and covered in a wet flat plate. The cells were incubated at 37 °C for 3 h to observe the degree of blood coagulation (Fan et al. 2021).

Determination of Peripheral White Blood Cell Count

The whole blood samples were collected by removing the eyeball of mice, and the blood samples were mixed evenly with anticoagulants to prevent blood coagulation. The white blood cell count was detected by the whole blood cell analyzer within 24 h (Park et al. 2018).

Assessment of Cytokines in Serum

Blood samples were obtained from the internal canthus vein of mice, centrifuged at 2000 r/min for 10 min, and the serum was collected and stored in a refrigerator at -80 ℃. Detection of cytokines based on TNF-*α*, IL-2, IL-6 Mouse ELISA kits using ELISA double antibody sandwich method (Cuellar-Nunez et al. 2021).

Data Statistics

In this experiment, the data were statistically analyzed by t-test, and the GraphPad Prism 7.0 software was used for comparison between groups. The data were expressed in the form of x ± SD, $P < 0.05$ is a significant difference, $P < 0.01$ is a highly significant difference, and $P > 0.05$ is a non-significant difference.

Results

Identification of Different Effective Parts of Ginseng

The flow chart of sample preparation is shown in (Fig. 1). Four different ginseng extract components, namely ginseng water extract (GWE), total ginsenoside complex (TGC), ginseng glycoprotein complex (GGC-1) and ginseng glycopeptide complex (GGC-2), were obtained by the following preparation process. DB 22/T 1668 - 2012: spectrophotometry, phenol sulfate method and BCA method were used to determine the purity of GWE more than 85.6%; DB 22/T 1668-2012: Spectrophotometric determination of TGC purity greater than 86.1%; the purity of GGC-1 was determined by phenol sulfate and BCA as > 88.4%; the purity of GGC-2 was determined by phenol sulfate and BCA as > 90.1%.

Body Weight and Immune Organ Index in Immunosuppressed Mice

As shown in (Fig. 2), the final weight of mice in each administration group showed an upward trend compared with NC. The thymus and spleen indexes of the MC group were significantly lower than those of the NC group ($P < 0.01$). The thymus index and spleen index of GWE, TGC, GGC-1 and GGC-2 groups were significantly higher than those of the MC group ($P < 0.01$).

Effect of Nonspecific Immune Function

Effect on Carbon Clearance. As can be seen from (Fig. 3A) that compared with NC, the phagocytic index of carbon clearance of immunosuppressed mice in the MC group was significantly decreased ($P < 0.05$). Compared with the MC group, the phagocytic indexes of rat carbon clearance in PC, GWE, TGC, GGC-1, and GGC-2 groups were significantly or extremely significantly increased ($P < 0.05$, $P < 0.01$). Compared with GWE, the phagocytic index of carbon clearance in TGC, GGC-1, and GGC-2 groups was significantly increased ($P < 0.01$).

Effect on NK Cell Activity. As shown in (Fig. 3B), NK cell activity was highly significantly reduced in the MC group compared to NC ($P < 0.01$). NK cell activity was highly significantly increased in the PC, GWE, TGC, GGC-1, and GGC-2 groups compared to the MC group ($P < 0.01$). There was no significant change in the TGC and GGC-2 groups compared to GWE ($P > 0.05$), and NK cell activity was significantly increased in the GGC-1 group of mice ($P < 0.01$).

Effects on Specific Immune Function

Effects on Cellular Immunity: Effect on Splenocyte Proliferation. As seen in (Fig. $4A_1$), the proliferation of splenocytes induced by ConA was significantly reduced in the MC group compared to NC ($P < 0.01$), and the proliferation capacity of splenic lymphocytes was highly significantly increased in the PC, GWE, TGC, GGC-1 and GGC-2 groups of mice compared to the MC group ($P < 0.01$). Compared with GWE, there was no significant change in the TGC group ($P > 0.05$), and the proliferation capacity of splenic lymphocytes was highly significantly increased in the GGC-1 and GGC-2 groups of mice ($P < 0.01$).

Effects on Cellular Immunity: Effect on Delayed Allergic Reaction. As seen in (Fig. $4A_2$), the degree of DTH was highly significantly reduced in the MC group compared to the NC group ($P < 0.01$). The degree of DTH was highly significantly increased in the PC, GWE, TGC, GGC-1, and GGC-2 groups compared to the MC group ($P < 0.01$). There was no significant change in the TGC group compared to the GWE ($P > 0.05$), and the degree of DTH was highly significantly increased in both the GGC-1 and GGC-2 groups of mice ($P < 0.01$).

Effect on Humoral Immunity: Effect on Serum Hemolysin Content. As shown in (Fig. 4B), mice in the MC group produced highly significant ($P < 0.01$) less hemolysin compared to the NC group. Mice in the PC, GWE, TGC, GGC-1, and GGC-2 groups all produced highly significant increases in hemolysin compared to the MC group ($P < 0.01$). Compared to GWE, mice in the TGC group produced a highly significant increase in hemolysin ($P < 0.01$), GGC-1 mice produced significantly more hemolysin ($P < 0.05$), and the GGC-2 group showed no significant change ($P > 0.05$).

Effect on the Total Peripheral Blood Leucocyte Count Assay

Leukocyte counts were significantly lower in the MC group than in the NC group (Fig. 5) ($P < 0.01$). The peripheral blood leukocyte counts of mice in the PC, GWE, TGC, GGC-1, and GGC-2 groups were all highly significantly higher compared to the MC group ($P < 0.01$). There was no significant change in the TGC group compared to GWE ($P > 0.05$), and the peripheral blood leukocyte count was significantly higher in the GGC-1 and GGC-2 groups of mice ($P < 0.05$, $P < 0.01$).

Effect on the Cytokines in Serum

As can be seen in (Fig. 6), the TNF-*a* content of mice in the MC group was highly significantly reduced compared with that in the NC group ($P < 0.01$). Compared with the MC group, the TNF-*a* content of mice in the GWE group was significantly increased ($P < 0.05$), and the TNF-*a* content of mice in the PC, TGC,

GGC-1, and GGC-2 groups was highly significantly increased ($P < 0.01$). Compared with GWE, TNF-α content was highly significantly increased in mice of TGC, GGC-1, and GGC-2 groups ($P < 0.01$); The IL-2 content of mice in the MC group was significantly lower than that of NC ($P < 0.01$). The IL-2 content of mice in the PC, GWE, TGC, GGC-1, and GGC-2 groups was highly significantly increased compared to the MC group ($P < 0.01$). Compared with the GWE group, the IL-2 content of mice in the TGC and GGC-1 groups was highly significantly decreased ($P < 0.01$), and there was no significant change in the IL-2 content of mice in the GGC-2 group ($P > 0.05$); The IL-6 content of mice in the MC group was highly significantly decreased compared to the NC group ($P < 0.01$). The mice in the PC, GWE, TGC, GGC-1, and GGC-2 groups all had a highly significant increase in IL-6 content compared to the MC group ($P < 0.01$). Compared with the GWE group, there was no significant change in the IL-6 content of mice in the TGC group ($P > 0.05$), a significant decrease in the IL-6 content of mice in the GGC-1 group ($P < 0.05$), and a highly significant decrease in the IL-6 content of mice in the GGC-2 group ($P < 0.01$).

Discussion

Single traditional Chinese medicine is composed of a large number of different components, and its function is very complex. Therefore, the known chemical components or components with large contents of a certain Chinese medicine are not necessarily the main pharmacodynamic components of this Chinese medicine. In addition, the components that play a major therapeutic role in a certain disease may also be different (Fan et al. 2019). In this study, CTX was selected to prepare immunosuppressive animal models to explore the effective parts of *Panax ginseng* C. A. Mey. that mainly have immunoregulatory effects. CTX has obvious damage to immune organs in the process of metabolism in vivo, which can directly act on cells to produce serious toxic effects and inhibit cellular and humoral immune responses and is widely used (Giuliani 2019, Xiang et al. 2020). And choose mushroom polysaccharide tablets which are commonly used in clinical to improve immunity as positive control drugs (Sheng et al. 2021).

By comparing the immunomodulatory effects of different effective parts of *Panax ginseng* C. A. Mey. on CTX immunosuppressive mice, it was found that the bodyweight of mice was not significantly decreased after continuous intragastric administration of ginseng water extract, ginseng total saponins, ginseng glycoprotein, and ginseng glycopeptide for 30 days, indicating that each administration group had no toxic effects on mice. The organ index was observed and it was found that different ginseng extracts in this experiment could reverse the decrease of immune organ index in mice induced by CTX to a certain extent. Since the thymus and spleen were the main immune organs involved in immune response, the relative weight of the thymus and spleen could preliminarily reflect whether the drugs affected immune organs and immune regulation, that is, the weight of the thymus and spleen would be relatively reduced due to the decrease of immune function (Li et al. 2017). Therefore, it was suggested that ginseng aqueous extract, ginsenosides, ginseng glycoproteins, and ginseng glycopeptides had protective effects on the two immune organs and could reverse the atrophy of immune organs induced by CTX.

The immune function includes non-specific immune function and specific immune function. The non-specific immune function includes the function of non-specific immune cells to remove body antigens.

Macrophages and NK cells are important components of non-specific immune cells in the immune system (Tomar and De 2014). Macrophages have the function of phagocytizing and clearing foreign bodies entering the body. Many immunoregulatory factors activate immune response according to the activity of macrophages, and the phagocytic rate used to respond to phagocytosis is one of the important indicators to measure nonspecific immunity (Nazimek and Bryniarski 2012). NK cells play a vital role in antiviral infection. They have a killing effect on a variety of tumor cells and target cells infected with the virus, without resensitization of antigens or participation of specific antigens or complements. Determination of NK cell activity is an important aspect of immune function (Abel et al. 2018, Iwasaki and Medzhitov 2015). In this study, it was found that ginseng water extract, total saponins of *Panax ginseng*, ginseng glycoproteins, and ginseng glycopeptides could enhance the mononuclear-macrophage function of mice to a certain extent and regulate the immune response by promoting NK cell activity in immunosuppressed mice induced by CTX. Among them, total saponins of *Panax ginseng*, and ginseng glycopeptides had good effects on improving the phagocytic function of macrophages, and ginseng glycoproteins had better effects on increasing the activity of NK cells.

Cellular and humoral immunity are important types of specific immune functions (Vazquez et al. 2015). In this study, the delayed-type hypersensitivity (DTH) induced by DNFB and the spleen lymphocyte transformation induced by ConA were used to determine the cellular immune function mediated by T cells. The determination of hemolysin can reflect the specific humoral immune function of the body. DTH specifically recognizes specific antigens by activated T lymphocytes, and then T lymphocytes proliferate and release cytokines (Sido 2018). After ConA stimulation, T lymphocytes in spleen cells undergo mother cell transformation, and their proliferation and activation determine the number of lymphocytes and affect the immune level, which is a common immune detection index. The Hemoagglutination method is to combine the two to form antigen-antibody complexes by adding SRBC to the serum-containing hemolysin and detecting the level of hemolysin by the degree of agglutination of SRBC (Ma et al. 2020). The results showed that all the extracted components could improve the inhibitory effect of cyclophosphamide on DTH reaction to a certain extent, promote the proliferation of T lymphocytes increase the production of antibodies in the serum of mice in coordination with ConA, and have the ability to activate cell and humoral immunity. Moreover, ginseng glycoprotein complex and ginseng glycopeptide complex had a better effect on cell immunity enhancement, and total ginsenoside complex and ginseng glycoprotein complex had a better effect on humoral immunity.

Leukocytes, as blood cells involved in the immune response, form antibodies, phagocytose foreign bodies, etc., and play an important role in the immune regulation of disease, the fight against many diseases, and the healing of body damage (Nourshargh and Alon 2014). This study found that ginseng water extract, total saponins of *Panax ginseng*, ginseng glycoprotein, and ginseng glycopeptide can promote the formation of white blood cells in CTX-induced immunosuppressive mice, and the effect of ginseng glycoprotein and ginseng glycopeptide is relatively more obvious.

Cytokines are an integral part of the body’s immune system, which can participate in the regulation of cell differentiation, proliferation, and activation. The amount of secretion directly reflects the immune status

of the body (Opal and DePalo 2000). Among them, TNF-*α* is an indispensable immunomodulatory factor for the body to maintain internal self-stability and resist various pathogenic factors. TNF-*α* has powerful anti-tumor effects, directly killing tumor cells and inhibiting DNA synthesis, activating macrophages, and increasing NK cell activity, and also plays an important role in the host defense mechanism (van Horssen et al. 2006). IL-2 promotes T cell growth, B cell proliferation, and differentiation, enhances the killing effect of cytotoxic T lymphocytes, and improves NK cell activity. IL-2 can induce the secretion of interferon and various cytokines and is a central part of immune regulation (Mitra and Leonard 2018). IL-6 is a multifunctional cytokine that regulates immune response, acute phase response, and hematopoietic function, and may play a central role in host defense mechanisms. It also plays a considerable role in improving T cell activation and B cell differentiation (Heinrich et al. 2003). The results of this study showed that different ginseng extracts were able to significantly increase the effect of relevant immune cytokines in mice, among which total ginsenoside complex, ginseng glycoprotein complex, and ginseng glycopeptide complex were more effective in increasing the content of TNF-*α*, ginseng water extract could better increase the content of IL-2, and ginseng water extract and total ginsenoside complex could effectively increase the content of IL-6 in mice.

Conclusions

The results of this study showed that different ginseng extracts were able to significantly increase the effect of relevant immune cytokines in mice, among which total ginsenoside complex, ginseng glycoprotein complex, and ginseng glycopeptide complex were more effective in increasing the content of TNF-*α*, ginseng water extract could better increase the content of IL-2, and ginseng water extract and total ginsenoside complex could effectively increase the content of IL-6 in mice.

Therefore, it can be inferred that ginseng water extract, total ginsenoside complex, ginseng glycoprotein complex, and ginseng glycopeptide complex, have the function of enhancing immunity, and ginseng glycoprotein is the main component that has the effect of improving immunity. It suggests that *Panax ginseng* C. A. Mey. has potential application prospects in the treatment of immunosuppressive diseases. The results of this experiment provide directional guidance for further research, which will be followed by studies on the structure of ginseng glycoproteins and the mechanism of their immune-modulating activity, providing a basis for the development of drugs and health food products with immune-enhancing effects as potential active ingredients and applications.

Declarations

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Author Contributions

Ping Yu, Qinghe Zhang and Changbao Chen designed the study. Ping Yu, Juntong Liu, Yuhan Zhang, and Yuqiu Chen collected the samples and performed the experiments. Ping Yu and Qinghe Zhang analyzed the data. Ping Yu wrote the manuscript. Qinghe Zhang revised the manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article.

Declarations

Conflicts of interest

Ping Yu declares that she has no confict of interest. Juntong Liu declares that she has no confict of interest. Yuhan Zhang declares that she has no confict of interest. Yuqiu Chen declares that she has no confict of interest. Qinghe Zhang declares that she has no confict of interest. Changbao Chen declares that he has no confict of interest.

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Figures

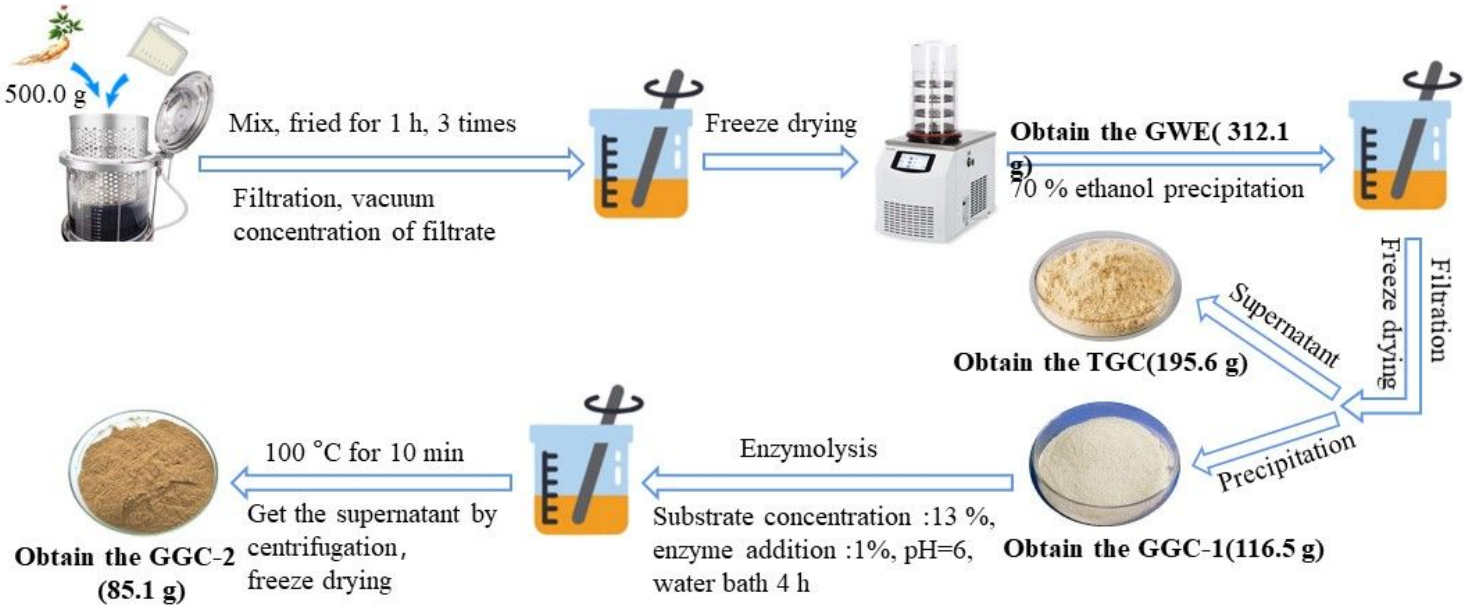

Figure 1

Preparation diagram of test product

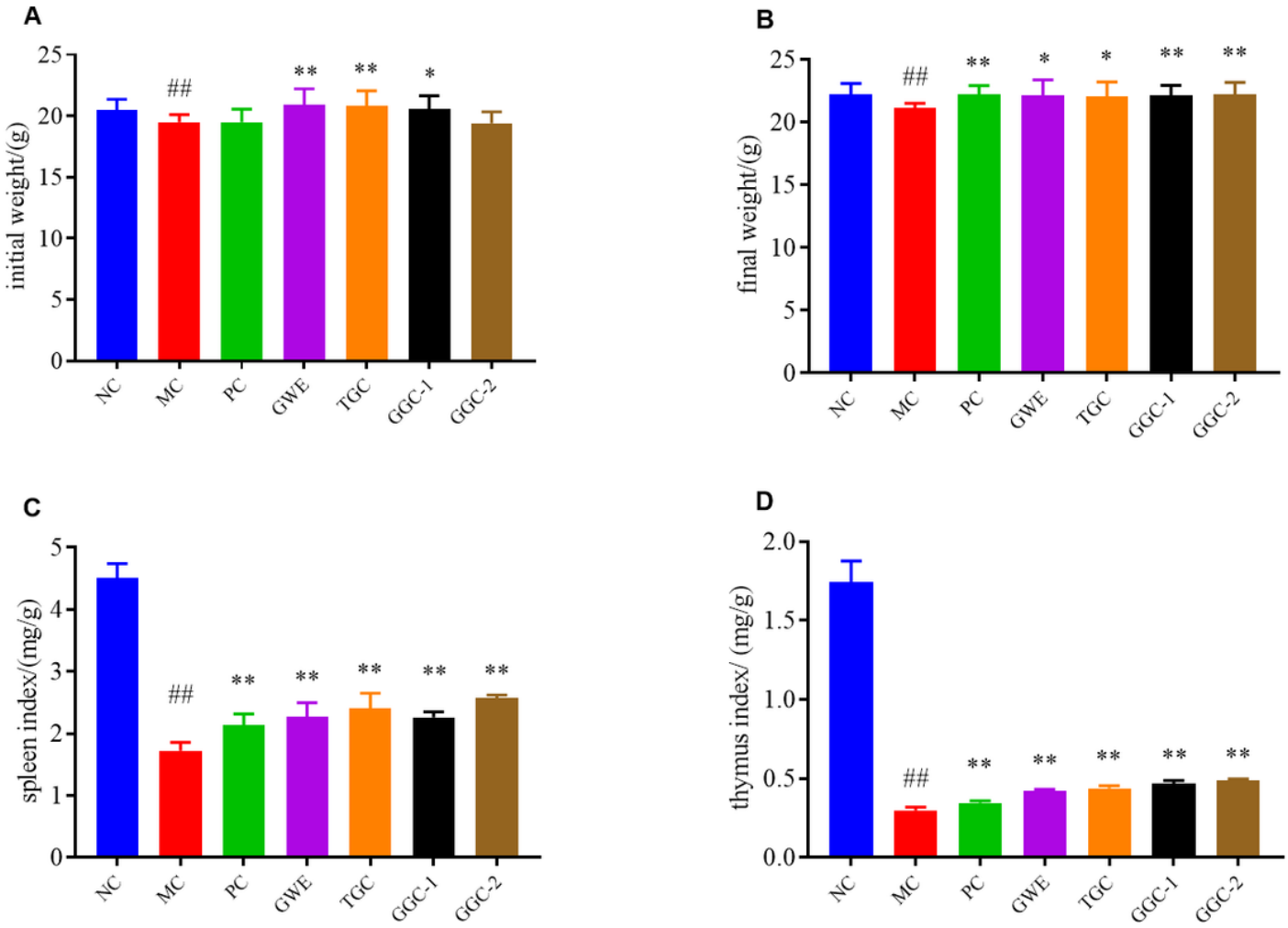

Figure 2

Experimental results of mouse body weight and immune organ index. (A) Initial weight. (B) Final weight. (C) Spleen index. (D) Thymus index. 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. Compare with NC group $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$; Compared with MC group * $P < 0.05$, ** $P < 0.01$.

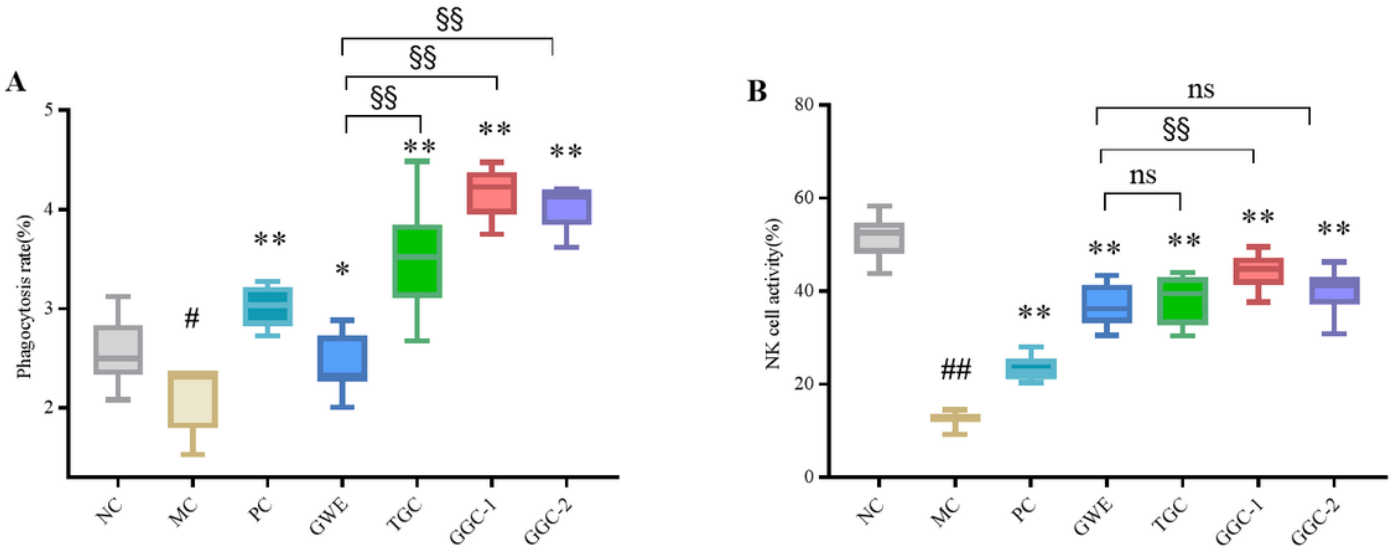

Figure 3

Effects of ginseng water extract (GWE), total ginsenoside complex (TGC), ginseng glycoprotein complex (GGC-1) and ginseng glycopeptide complex (GGC-2) on (A) macrophages activation, (B) 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 NC group; * $P < 0.05$, ** $P < 0.01$ when compared to MC group; § $P < 0.05$, §§ $P < 0.01$, ^{ns} $P > 0.05$ when compared to GWE group.

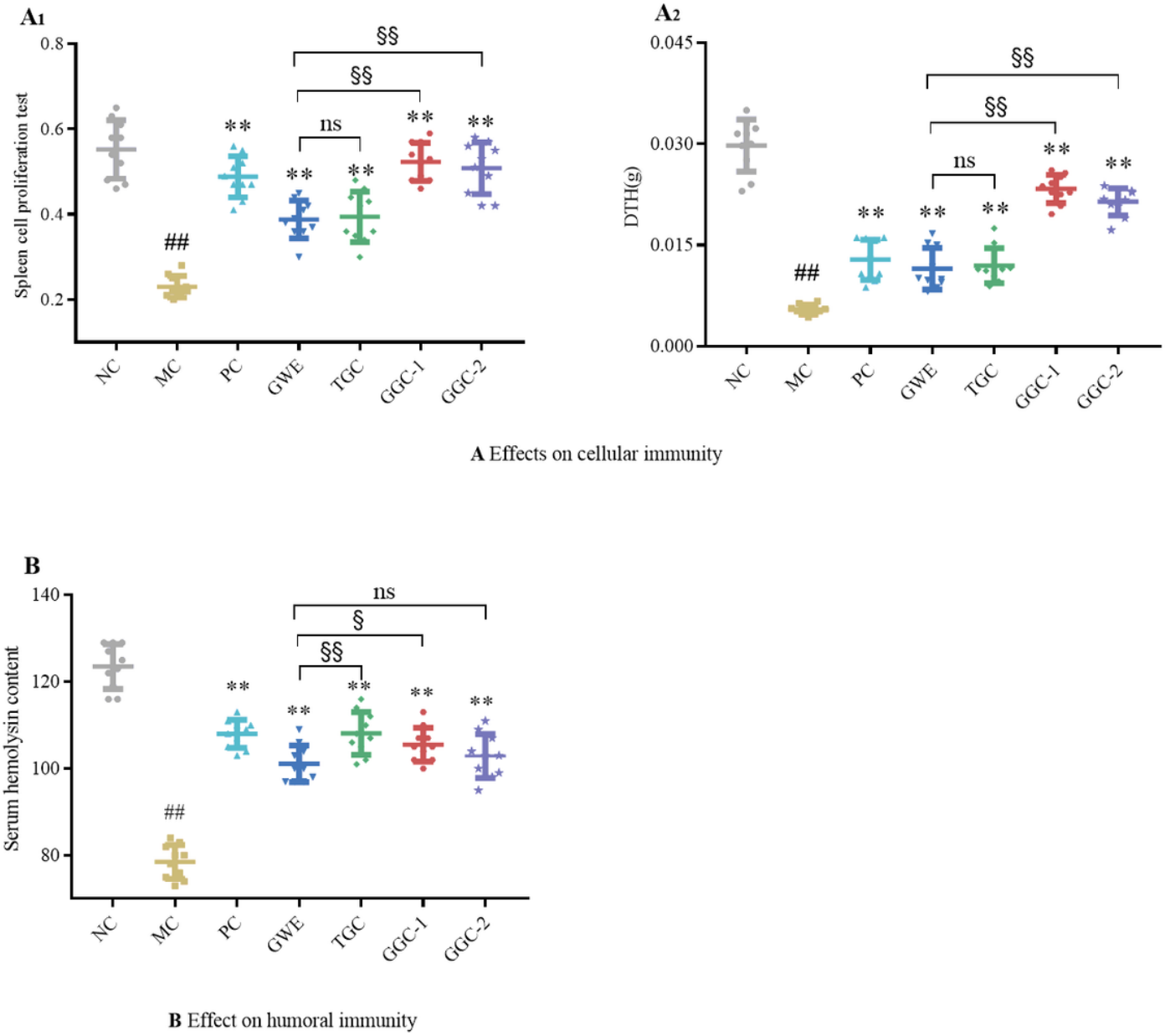

Figure 4

Effects of ginseng water extract (GWE), total ginsenoside complex (TGC), ginseng glycoprotein complex (GGC-1) and ginseng glycopeptide complex (GGC-2) on (A_1) splenocyte proliferation, (A_2) DNFB-induced DTH, (**B**) serum hemolysin. Results are given as Mean ± SD (n = 10) in each group. Significance at $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ when compared to NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to MC group; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{ns}P > 0.05$ when compared to GWE group.

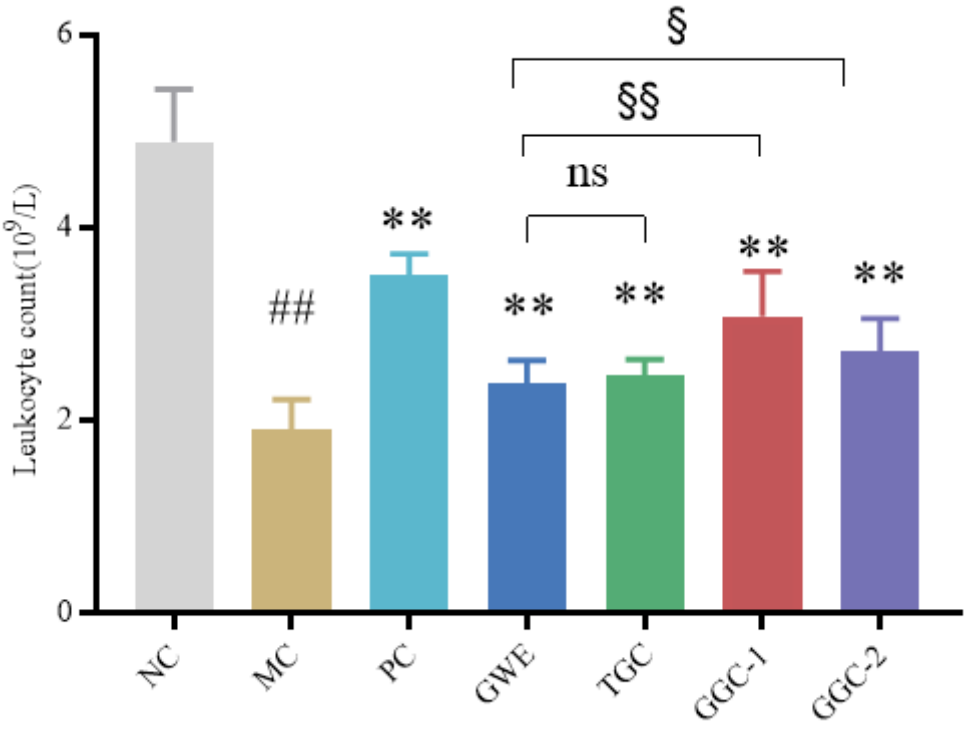

Figure 5

Effects of ginseng water extract (GWE), total ginsenoside complex (TGC), ginseng glycoprotein complex (GGC-1) and ginseng glycopeptide complex (GGC-2) on leukocyte count. Results are given as Mean ± SD (n = 10) in each group. Significance at $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ when compared to NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to MC group; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{ns}P > 0.05$ when compared to GWE group.

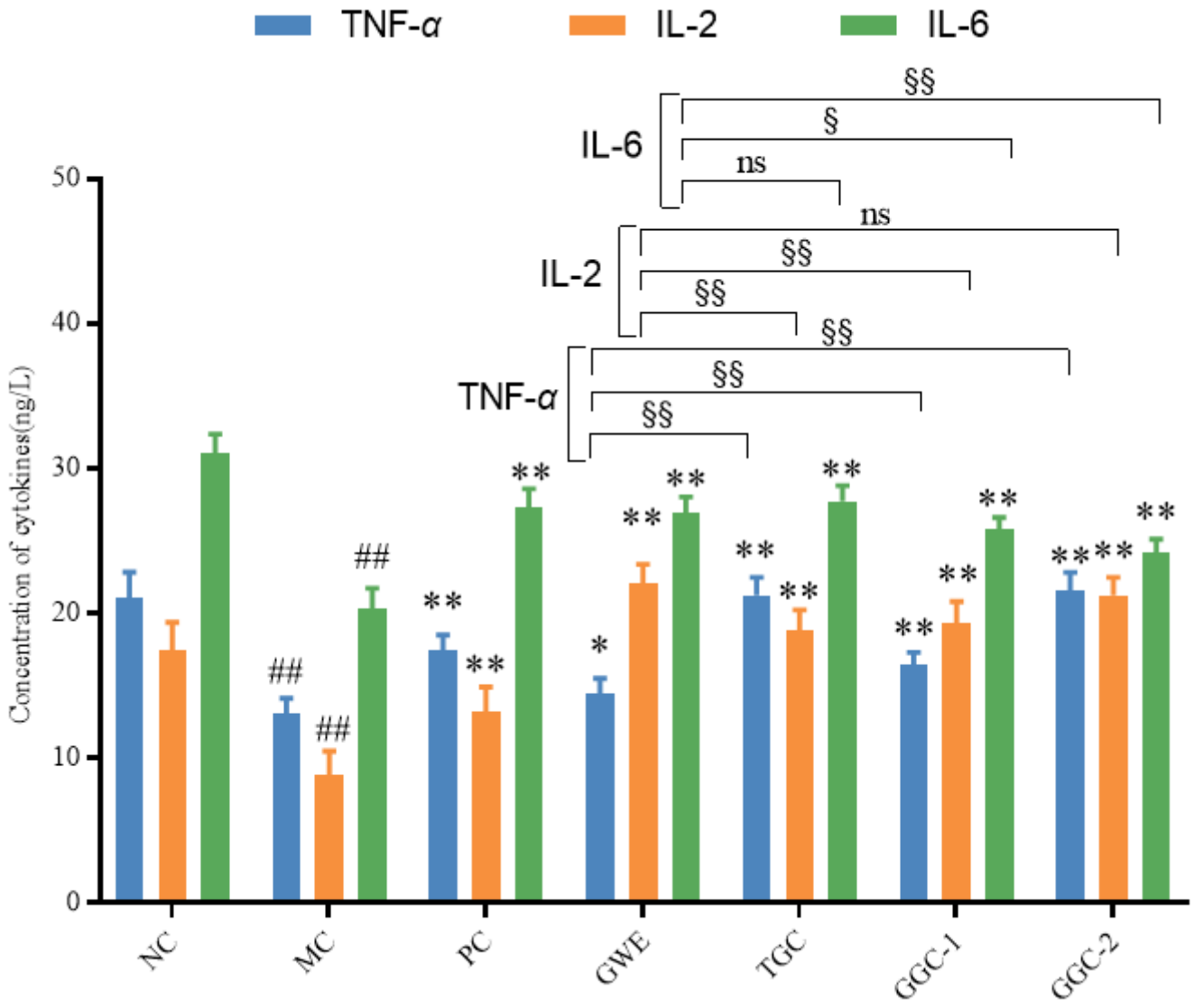

Figure 6

Effects of ginseng water extract (GWE), total ginsenoside complex (TGC), ginseng glycoprotein complex (GGC-1) and ginseng glycopeptide complex (GGC-2) on cytokines in Serum. Results are given as Mean ± SD (n = 10) in each group. Significance at $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ when compared to NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to MC group; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{ns}P > 0.05$ when compared to GWE group.