The Protective Effects of Three Polysaccharides From Abrus cantoniensis Against Cyclophosphamide-Induced Immunosuppression and Oxidative Damage

Dongshuai Qu1†, Hongjie Hu1†, Shuaitao Lian1, Wenjing Sun2* and Hongbin Si1*

1 State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Animal Science and Technology, Guangxi University, Nanning, China, 2 Guangxi Key Laboratory of Agricultural Resources Chemistry and Biotechnology, College of Biology & Pharmacy, Yulin Normal University, Yulin, China

This study was designed to systematically elucidate the immunomodulatory and antioxidant effects of three polysaccharide fractions (ACP_{60}, ACP_{80}, and ACP_{12}) from Abrus cantoniensis on cyclophosphamide (CTX)-induced immunosuppressive mice. The experimental mice were divided into 12 groups, then modeled and administrated with different doses of three polysaccharides (50, 150, 300 mg/kg/day) by gavage. The results showed that ACP could markedly recover the CTX-induced decline in immune organ and hemocytes indexes and promote proliferation of splenocytes, earlap swelling rate, secretion of cytokines (TNF-α, IFN-γ, IL-1β, IL-6), and immunoglobulin (Ig-M and Ig-G). Additionally, ACP improved the enzymatic activities of T-SOD and GSH-PX greatly, while the level of MDA was significantly decreased in the liver. In particular, ACP_{12} had higher immunomodulatory and antioxidant activities than ACP_{60} and ACP_{80}. Based on the present findings, ACP could be utilized as an efficacious candidate for immunomodulators and antioxidants, which provide a new application prospect in the food and pharmaceutical industries.

Keywords: immunoregulation, antioxidation, natural polysaccharides, cyclophosphamide-induced immunosuppression (CTX-induced immunosuppression), Abrus cantoniensis

INTRODUCTION

Abrus cantoniensis, which belongs to the Abrus genus in the Leguminosae family, is mainly distributed in southern China, particularly in Guangdong, Guangxi, and Hunan provinces (1). This native plant has been commonly used as folk herbal medicine for many types of diseases or as a functional food for healthcare for centuries. It is reported that A. cantoniensis is usually used in health care food, soups, herbal tea, or beverages, such as tortoise jelly (Gui Ling-Gao), the red-canned tea “Wong Lo Kat,” and some Chinese patent medicines, such as the well-known “Jigucao capsules.” Extensive studies have indicated that A. cantoniensis possesses the protection against many physical illnesses such as jaundice, hepatitis, cirrhosis with ascites (2), gastric ulcer (3), sore throat (4), rheumatism, blood stasis, and internal injuries (5), and treatment of wound in vivo (6).
It is well documented that the effective ingredient of *A. cantoniensis* contains polysaccharides, flavonoids, phenolic compounds, alkaloids, terpenoids, saponins, and sapogenols (7). Polysaccharides, which are ubiquitous macromolecules in nature, have attracted great attention owing to their anti-oxidant (8), anti-tumor, immunomodulatory activity (9), and hypoglycemic and hypolipidemic (10) with non-toxicity and unnoticeable side effects. The immunomodulation and antioxidant properties are the most attractive bioactivities of polysaccharides. Polysaccharides derived from *Cordyceps militaris* could improve immunity and antioxidant activities (11). Some researchers found that *Desmodesmus armatus* polysaccharides can improve the antioxidant capacity in a variety of ways (12). Therefore, it is meaningful to explore the immunomodulatory effects and antioxidant activities of *A. cantoniensis* polysaccharide (ACP).

The immune system is responsible for recognizing and clearing antigenic foreign bodies through specific and non-specific immunity under normal physiological conditions, which aim to maintain an immune homeostasis balance. However, this balance can be affected and destroyed by numerous factors, such as malnutrition, infectious disease, and stressors. Furthermore, the immune imbalance will further lead to a variety of diseases (13, 14). Cyclophosphamide (CTX), a potent immunosuppressant in clinical chemotherapy, can cause severe side effects, such as damaging immune organs, avoiding the production of cytokines and antibodies, and inhibiting the proliferation of T and B lymphocytes (15). Therefore, CTX is often used as a drug to establish immunocompromised mouse models (16–18).

In our previous study, ACP has been proved to have favorable immunomodulation and antioxidant *in vitro*. However, these capacities and the key fraction of ACP in target animals have not been confirmed until now. The purpose of this investigation is to explore the immunomodulatory effect and antioxidant activity of different fractions of *ACP in vivo*. In the present study, we isolated and purified three polysaccharide fractions from *A. cantoniensis* and investigated their physicochemical features. The mice were immunosuppressed intraperitoneal injection of CTX, and then the immune indexes and antioxidant enzyme activity were evaluated and compared by gavage administration of different concentrations of three polysaccharide fractions.

### MATERIALS AND METHODS

#### Plant Materials and Chemicals

*A. cantoniensis* was purchased from Guangxi Dahong Pharmaceutical Co., Ltd. (Hechi, Guangxi, China), and then was crushed by a disintegrator (800Y, Yongkang Bouu Hardware Products Co., Ltd.).

Cyclophosphamide was obtained from the Shanghai Beyotime Biotechnology Co., Ltd. dinitrofluorobenzene (DNFB) was obtained by Macklin (Shanghai, China). The ELISA kits of mouse tumor necrosis factor (TNF-α), interferon (INF-γ), interleukin (IL-6, IL-1β), Immunoglobulin M (Ig-M), Immunoglobulin G (Ig-G), and the assay kits for malondialdehyde (MDA), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px) were acquired from Jiangsu Jingmei Biotechnology Co., Ltd (Jiangsu, China). The cell culture medium RPMI1640, Astragalus polysaccharides (APS), and MTT were supplied from Solarbio (Beijing, China).

#### Extraction and Purification of Polysaccharides

Briefly, *A. cantoniensis* was pretreated with 95% ethanol to remove pigment for reserve use. The crude polysaccharides of *A. cantoniensis* were isolated by the water–ethanol extraction method. After the proteins were removed by the Sevag method, anhydrous ethanol was added to the extracts to make the concentration of ethanol in the system reach 80% with light stirring. After alcohol precipitation at 4°C overnight, the resulting precipitate was redissolved in ultra-pure water, and then further purified by DEAE-52 anion exchange chromatography (2.6 × 50 cm²). The resulting solution was eluted with ultrapure water and 0.2 M NaCl into two peaks, the eluents were concentrated and dialyzed (cut-off MW3,500 Da) against distilled water for 36 h and vacuum-dried under freezing to obtain the refined ACP₀ and ACP₁₂ respectively.

In addition, the concentrated extract was added with anhydrous ethanol, and the concentration of ethanol in the system reach 40%. After centrifugation, the precipitation was collected and freeze-dried, namely, ACP₆₀. According to the above operation, ethanol was added to make the final concentration of ethanol in the system reach 60 and 80% in turn; ACP₆₀ and ACP₈₀ were obtained, respectively. Previous *in vitro* experiments showed that ACP₆₀, ACP₈₀, and ACP₁₂ have excellent effects on antioxidant activity and immunomodulatory effects. Therefore, these three polysaccharide fractions were selected for further study.

#### General Methods

The total carbohydrate content was determined by the phenol–H₂SO₄ method using glucose as a standard (19). The total uronic acid content was determined by the carbazole sulfate method using glucuronic acid as a standard (20). Protein content was measured by the Coomassie brilliant blue method using bovine serum albumin as a standard. Visible absorbance was recorded with a UV–Vis spectrophotometer (Model SP-752, China).

The ultraviolet absorbance of the ACP aqueous solution (1 mg/ml) was scanned by a U-6000PC spectrophotometer (Yuanxi, Shanghai, China) in the range of 200–600 nm. Fourier-Transform Infrared Spectroscopy (FTIR) analysis, ACP (2 mg) was ground with KBr and obtained in the range of 400–4,000/cm on a Thermo Nicolet iS5 FTIR (ThermoFisher, USA).

The molecular weight (MW) distributions of the polysaccharide samples were determined using High-Performance Gel Permeation Chromatography (HPGPC) on a TSK-GEL GMPWXL column (7.5 mm × 300 mm, 10 µm; Tosoh, Tokyo, Japan), and the monosaccharide compositions of the polysaccharide samples were analyzed by High-Performance Liquid Chromatography (HPLC) using a Thermo C18 column (250 × 4.6 mm) with 1-phenyl-3-methyl-5-pyrazolone (PMP) pre-column derivatization. The HPGPC and HPLC were carried out by using a Dionex Ultimate 3000 Liquid chromatography...
(ThermoFisher, USA) and a 1260 Liquid chromatograph (Agilent, California, USA).

**Animals and Experimental Design**

Specific-pathogen-free (SPF) Female Kunming mice (20–22 g) were provided by Changsha Tianqin Biotechnology Co., Ltd., China (certificate number: SCXK(Xiang)2019–0014, Hunan). Before and during experiments, all of the animals were housed in pathogen-free cages with wood shavings in a room (temperature 25 ± 2°C, relative humidity 65 ± 5%) with a 12 h/12 h light–dark cycle. The mice were fed with distilled water and rodent chow ad libitum. All animal experimental procedures were approved by the Animal Experimental Ethics Committee of Guangxi University.

After 1 week of acclimatization, the mice were divided into 12 groups randomly (each group \( n = 10 \)): normal group, model group, positive group, ACP\(_{60}\) low-dose group, ACP\(_{60}\) medium-dose group, ACP\(_{60}\) high-dose group, ACP\(_{80}\) low-dose group, ACP\(_{80}\) medium-dose group, ACP\(_{80}\) high-dose group, ACP\(_{12}\) low-dose group, ACP\(_{12}\) medium-dose group, and ACP\(_{12}\) high-dose group. The positive group was fed APS (150 mg/kg/day), and the normal group and model group were administrated 0.2 ml saline (0.1 ml/10 g) via gastric gavage once daily. 50/150/300 mg ACP\(_{60}/kg/day\) per mouse for ACP\(_{60}\)-L, ACP\(_{60}\)-M, ACP\(_{60}\)-H groups; 50/150/300 mg ACP\(_{80}/kg/day\) per mouse for ACP\(_{80}\)-L, ACP\(_{80}\)-M, ACP\(_{80}\)-H groups; 50/150/300 mg ACP\(_{12}/kg/day\) per mouse for ACP\(_{12}\)-L, ACP\(_{12}\)-M, ACP\(_{12}\)-H groups. The experimental procedure was implemented for 21 consecutive days. From Day 15 to Day 17, except for the normal group, all of the mice were intraperitoneally injected with 80 mg/kg/day CTX to form the immunosuppressive model. Twelve hours after the last administration, all the mice were weighed and then sacrificed by cervical dislocation to obtain blood and organs. The experimental design is outlined in Figure 1.

**Analysis of Immune Organ Indexes**

After experimental mice were sacrificed by cervical dislocation, the spleen and thymus were isolated surgically under sterile conditions and weighed immediately. The immune organ indexes were calculated according to the following formula:

\[
\text{The immune organ indexes (mg/g) = weight of spleen or thymus (mg)/body weight (g)}
\]

**Delayed-Type Hypersensitivity Reaction**

On Day 16 of the experimental procedure, barium sulfide was used to shave belly fur off of each mouse with a range of about 2.0 cm × 2.0 cm, and then they were painted evenly with 50 µl 1% DNFB for sensitization. Five days later, 10 µl 1% DNFB was evenly smeared on both sides of the right earlap of each mouse. Approximately 24 h after the attack, they were executed. The ear slices (8 mm diameter) of both earlaps of each mouse were removed by puncher and weighed. The degree of DTH is reflected by the weight differentials between the right and left ear slices of each mouse.

**Assay of Splenocyte Proliferation**

The splenic lymphocytes were obtained from the mouse spleen and the MTT assay was used to determine T and B lymphocyte proliferative capabilities induced by ConA and LPS, respectively. Briefly, the mice spleens were removed from sacrificed mice under germ-free conditions, and then chopped, ground, washed, and filtered to get a homogeneous cell suspension of splenocytes. The suspension was centrifuged at 2,500 rpm for 5 min and the precipitate was treated with an erythrocyte lysis solution. Subsequently, the splenocytes were washed two times by PBS and resuspended in RPMI 1640 medium with 10% newborn bovine serum. The cells were adjusted to the final density of \( 1 \times 10^6 \) cells/ml. Suspension of splenocytes (200 µl) was placed into a 96-well microtiter plate, with LPS (10 µg/ml) or Con A (10 µg/ml). Serum-free RPMI 1640 medium was used as the control, then cultured at 37°C with 5% CO\(_2\). After 44 h of incubation, 20 µl of MTT solution (5 mg/ml) was added to each well and then continued to incubate for 4 h. Then the supernatant was removed and we added 150 µl DMSO solution into each well. Finally, the optical density (OD) was recorded using a Microplate Reader at 570 nm (Thermo Fisher, USA).

**Effect of ACP on WBC, LYM, RBC, HGB, and PLT Levels**

After experimental mice were executed, blood samples were collected by enucleating eyeball and 200 µl of which was placed into an anticoagulant tube. The contents of white blood cell (WBC), lymph (LYM), hemoglobin (HGB), red blood cell (RBC), and platelet (PLT) were measured by a Hematology Analyzer (URIT-5160Vet). The other blood was placed into a centrifugal tube and exerted to obtain the serum.

**Assay Release of Cytokine and Quantity of Serum Immunoglobulins**

Blood samples in each group were collected from the ophthalmic venous plexus and centrifuged at 3,500 rpm for 15 min at 4°C to get the serum. Subsequently, the serum was collected to determine the levels of cytokines (TNF-\( \alpha \), IFN-\( \gamma \), IL-1\( \beta \), and IL-6) and immunoglobulins (IgG and IgM) using the ELISA kit according to the manufacturer’s protocols.

**Effect of ACP on Activities of Antioxidant Enzymes**

The same wet weight liver was taken in ice-cold normal saline. The liver tissue homogenate was prepared by an automatic homogenizer, and then, centrifuged at 2,500 rpm for 10 min at 4°C. The supernatant was collected to measure the levels of T-SOD, GSH-PX, and MDA with commercial kits.

**RESULTS**

**Physicochemical Features of ACP**

ACP was obtained through water extraction, ethanol precipitation, deproteination, DEAE-cellulose anion exchange chromatography, dialysis, and lyophilization. These results revealed that the total sugar content of ACP\(_{12}\) (99.6%) was higher than ACP\(_{60}\) (89.8%) or ACP\(_{80}\) (86.1%). Due to removing protein
FIGURE 1 | Schematic diagram of the experimental design. ACP, Abrus cantoniensis polysaccharides; CTX, cyclophosphamide.

TABLE 1 | The total sugar, uronic acid, and protein contents, and monosaccharide composition molecular weight of the ACP.

| Fraction | Total sugar (%) | Uronic acid (%) | Protein (%) | MW (kDa) | Monosaccharide composition (%) |
|----------|----------------|----------------|-------------|---------|-------------------------------|
| ACP<sub>60</sub> | 60.8 | 38.4 | 0.05 | 86.9 | Man% 3.4, GlcN% 12.1, Rha% 1, GlcUA% 1.2, GalUA% 8.0, Glc% 71.5, Gal% 9.6, Xyl% 2.0, Ara% 3.8 |
| ACP<sub>80</sub> | 86.1 | 15.5 | 0.16 | 26.2 | 5.7, 1.0, 1.7, 1.7, 4.9, 53.5, 14.3, 0.2, 16.8 |
| ACP<sub>t2</sub> | 99.6 | 27.9 | 0.11 | 145.6/8.9 | 8.8, 1.7, 3.9, 22.2, 11.0, 25.6, 6.3, 16.6 |

by the Sevag method and DEAE–cellulose anion exchange chromatography, the protein content of ACP<sub>60</sub>, ACP<sub>80</sub>, and ACP<sub>t2</sub> were only 0.05%, 0.16%, and 0.11%, respectively (Table 1), which was consistent with its absorption at 280 nm in the UV spectrum (Figure 2). Additionally, there was no peak at 260 nm of UV spectra, signifying the absence of nucleic acid.

The Carbazole sulfate method indicated that the uronic acid contents of ACP<sub>60</sub>, ACP<sub>80</sub>, and ACP<sub>t2</sub> were 27.9%, 38.4%, and 15.5%, respectively (Table 1). The monosaccharide composition analysis indicated that ACP<sub>t2</sub> was mainly composed of galactose (25.6%), galactose uronic acid (22.2%), arabinose (16.6%), glucose (11.0%), and a small amount of mannose, xylose, galactose uronic acid, and rhamnose. ACP<sub>80</sub> mainly contains glucose (53.5%), arabinose (16.8%), and galactose (14.3%), while ACP<sub>60</sub> mainly consisted of glucose (71.5%), galactose (9.6%), and galactose uronic acid (8.0%; Table 1).

High-performance gel permeation chromatography analysis showed that ACP<sub>t2</sub> exhibited two main elution peaks. Based on known standard polysaccharides, the MW of two peaks was calculated at 145.6 and 8.9 kDa. ACP<sub>60</sub> and ACP<sub>80</sub> both exhibited one main elution peak: the molecular weight was estimated at MW 86.9 and 26.2 kDa, respectively.

Fourier-Transform Infrared Spectroscopy spectra of the three polysaccharides fractions are shown in Figure 2. The results indicated that the characteristic peak was at 3,400, 2,930, and 1,420/cm, which is caused by O–H, C–H, and C–O stretching vibrations, respectively (21). These characteristic absorption peaks are often used to identify polysaccharide samples. Additionally, 1,750 and 1,610/cm were probably caused by the C=O or bound water stretching vibration (22). The prominent peaks in the range of 1,000 to 1,200/cm (1,123.78, 1,027.02, and 1,025.08/cm) corresponded to C–H variable angle telescopic vibration, indicating the existence of a pyranose- ring structure in ACP<sub>60</sub> and ACP<sub>80</sub> (23). The band at 1,238/cm might represent the O=S=O deformation vibration, indicating that sulfates were present in the ACP<sub>t2</sub>. The absorption peak of 1,076.98/cm might represent C–O–H or C–O–C stretching vibrations. The modifications of characteristic groups will lead to changes in the physical and chemical properties of the polysaccharide, thus causing different biological activities.

Effect of ACP on Immune Organs in Immunosuppressed Mice

As shown in Figures 3A,B, the mice experiment was designed to examine the effect of CTX on immune organs and improvements mediated by three polysaccharide fractions. The thymus and spleen are the main immune organs in the body, thus immune function and prognosis of an organism could be reflected by
FIGURE 2 | UV spectrum and FTIR spectra of ACP_{60}, ACP_{80}, and ACP_{12}.
FIGURE 3 | (A) Effect of ACP60, ACP80, and ACP12 on spleen index in CTX-treated mice. (B) Effect of ACP60, ACP80, and ACP12 on thymus index in CTX-treated mice. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01 vs. the normal group. #P < 0.05, ##P < 0.01 vs. the model group.

The indexes of immune organs modestly (24, 25). The results showed that compared with the normal group, the indexes of the spleen and thymus in the model group radically declined (P < 0.01), indicating that the immunosuppressive model was established preliminarily. Supplementation with different doses of polysaccharides, the spleen and thymus indexes rose in all three polysaccharides-treated mice in varying degrees compared with the model group. Especially in the ACP12 group, the dose of 150 mg/kg resulted in the most significant effect. These results demonstrated that ACP60, ACP80, and ACP12 could improve the damage to immune organs of mice induced by CTX and enhance immunity. ACP12 was associated with the strongest immunomodulatory effect.

Delayed-Type Hypersensitivity Reaction

Compared with the normal group, the earlap swelling rate of model mice was significantly decreased (P < 0.01; Table 2), indicating that CTX damaged the cellular immunity of mice seriously. After treatment with ACP60, ACP80, ACP12, and APS (positive control), the earlap swelling rate in all the groups was increased to different degrees in a dose-dependent manner compared with the model group. There was no remarkable difference in the swelling rate between the ACP80-H ACP12-M group, ACP12-H group, and normal group (P > 0.05). ACP12 at the dose of 150 mg/kg resulted in the strongest recovery function. The results showed that three polysaccharide fractions can increase the cellular immunity of mice by enhancing the degree of earlap swelling.

Effect of ACP on Splenic Lymphocyte Proliferation in Immunosuppressed Mice

As shown in Figures 4A,B, compared with the normal group, the proliferation of T and B lymphocytes (43.94 and 58.20%, respectively) in the CTX-treated group declined (P < 0.01), which showed cellular and humoral immune functions of immunosuppressed mice were impaired. With treatment of three polysaccharide fractions, a decline in the proliferation of T and B lymphocytes was alleviated in a dose-dependent
manner. Importantly, the lymphocyte proliferation rates of mice treated with the ACP12 group (50, 150, 300 mg/kg) were marked higher than those of mice treated with ACP60 and ACP80 groups. The results of the proliferation assay indicated that three polysaccharide fractions had the potential for promoting the activation and proliferation of T and B lymphocytes and then enhancing the cellular and humoral immune functions of immunosuppressed mice. Similarly, positive control (150 mg/kg) showed a similar effect.

**Effect of ACP on WBC, LYM, RBC, HGB, and PLT Levels**

As shown in Table 3, compared with the normal group, the levels of WBC, LYM, RBC, HGB, and PLT in peripheral blood of the model group were lower after intraperitoneal injection of CTX, which indicates that injection of CTX seriously damaged the health of mice. However, following oral administration of three polysaccharide fractions, these CTX-induced decreases were ameliorated (P < 0.05). Compared with the model group, the levels of ACP80-M, ACP80-H, ACP12-L, ACP12-M, ACP12-H group, and the positive group increased significantly (P < 0.05, P < 0.01) and the effects of ACP80-H, ACP12-M, and ACP12-H groups were stronger than those of the positive group by oral administration of APS (P < 0.05). The effects of the same dose of ACP60 and ACP80 groups were remarkably weaker than those of the ACP12 group (P < 0.05). It is suggested that ACP can alleviate the decrease of levels of blood cells caused by CTX, and ACP12 is more effective than ACP60 and ACP80.

**Effect of ACP on Serum Levels of Cytokines and Immunoproteins in Immunosuppressed Mice**

The changes in cytokine concentrations in serum are shown in Figures 5A–D. After CTX injection, the concentrations of TNF-α, IFN-γ, IL-1β, and IL-6 in serum were significantly decreased (P < 0.01). After administration of three polysaccharides, the levels of TNF-α and INF-γ increased in a dose-dependent manner, and the concentrations of TNF-α and IFN-γ in the ACP12-M group and ACP12-H group were not significantly different compared with the normal group (P > 0.05). The concentrations of IL-1β and IL-6 also showed similar results, in which the effect of the medium dose of ACP12 was stronger than that of the high dose of ACP12. It may be due to the overburden of a high dose of ACP12 on mice, resulting in mild toxicity. The recovery effect of ACP12 groups on cytokines was stronger than that of ACP60 and ACP80 groups, which indicated that ACP12 had a significant enhancing effect on the immune activity of immunosuppressed mice.

As shown in Figures 5E,F the serum IgG and IgM concentrations from the model group were lower than those of the normal group as expected (P < 0.01). Compared with the model group, the contents of IgG of the three polysaccharides fractions were significantly higher (P < 0.05, P < 0.01), while the content of IgM of ACP80-M, ACP80-H, and all ACP12 groups significantly increased (P < 0.05). Based on current results, it was implied that ACP80 and ACP12 can directly affect the strength of the immune function.

**Effect of ACP on Antioxidant Activities in Immunosuppressed Mice**

As shown in Figure 6, the levels of T-SOD and GSH-PX in the model group declined significantly compared with the normal group (P < 0.01), while the concentration of MDA in the liver of model mice presented a significant increase to 13.36 ± 0.45 nmol/L (P < 0.01). Compared with the model group, the activities of T-SOD and GSH-PX in all three polysaccharide groups and the positive group were increased to different degrees. The activities of T-SOD and GSH-PX in the ACP12-M group and ACP12-H group were significantly stronger than that of the positive control group (P < 0.05). At the same dose, the recovery of T-SOD and GSH-Px activities in ACP12 groups was significantly stronger than that in ACP60 and ACP80 groups (P < 0.05). The content of MDA in the liver decreased with the increase of the concentration of sample polysaccharides. Compared with the ACP12-M group, the decreasing trend of MDA content in the ACP12-H group stopped, and it was speculated that ACP12 had an inhibitory effect due to excessive dose. These results indicated that injection of CTX seriously destroyed the antioxidant ability of the experimental mice. However, three polysaccharides can increase the activities of T-SOD and GSH-Px and decrease the content of MDA in immunosuppressive mice. In particular, ACP12 was associated with the largest effect on antioxidant ability.

**DISCUSSION**

Many factors (nutrition, disease, environment, and others) leave the body in an immunosuppressed state, which increases susceptibility to secondary infections and reduces the effectiveness of vaccines and drugs (26). However, the cellular and humoral immune mechanisms involved in the body's immune system are very complex. Host protection from immune damage and oxidative damage in the immunosuppression state...
is a problem worthy of attention. Immunomodulators play an important role in immune function, many herbal-based immunomodulators (such as polysaccharides, flavonoids) can enhance the host defense response, which is an effective way to improve the resistance of animals to various adverse factors (27–29). For hundreds of years, the *A. cantoniensis* has attracted wide attention for its range of biological activities. Meanwhile, the protective effect of ACP on host immunosuppression and oxidative damage has not been reported. In this study, CTX was used to establish a mouse immunosuppression model to study the regulation of ACP on immune function and the protective effect of oxidative damage in mice.

As the main immune organs, the thymus and spleen can directly affect the level of immunity which are the locations where immune cells grow and proliferate (13). Studies have shown that polysaccharides from *Mulberry leaf* can restore the immune organ atrophy induced by CTX, thus promoting the development of the immune system (30). In this study, after dealing with CTX by intraperitoneal injection, the spleen and thymus indexes of mice came down markedly, indicating that the immunosuppressive model was established successfully. All three natural polysaccharides increased these indexes, suggesting that polysaccharides can protect the body’s immune organs and thus improve the host’s immune function.

Lymphocytes are the core cells in the immune system. It plays a crucial role in humoral immunity and cellular immunity, mainly undertaking the task of the acquired immune response. Lymphocytes can be activated after the process of antigen presentation and mitogen stimulation (31). T lymphocyte is the main component of cellular immunity, which could directly inhibit and destruct target antigens, such as tumor cells and viruses. In humoral immunity, the corresponding antigen could activate B lymphocyte, which is differentiated into plasma cells to produce a variety of antibodies (32). The proliferation
process of T lymphocyte was induced by ConA (T-cell mitogen) and B lymphocyte was induced by LPS (B-cell mitogen), which aims to evaluate the functions of cellular immunity and humoral immunity. Previous studies have shown that natural polysaccharides can significantly improve splenic lymphocyte proliferation in immunosuppressed mice induced by CTX. Zhou et al. obtained a polysaccharide from Lonicera japonica (LJP) and Lonicera japonica (LJP) and found that LJP (60,120 mg/kg) could significantly improve the proliferation of spleen lymphocytes in immunosuppressed mice induced by CTX (14). Li et al. (33) found that different doses of GFP-2 combined with ConA or LPS significantly increased the proliferation of T and B lymphocytes, and there was a significant dose correlation. This suggests that polysaccharides protect the mouse immune system effectively.

Hemocyte includes WBC, LYM, RBC, HGB, and PLT. Immune-related genes could be expressed in hemocytes. Many immunocompromised diseases or infectious diseases are closely related to the number of WBCs (25). Lymphocytes participate in cellular and humoral immunity of the body (38, 39). Red blood cells can also play a role in immune response, such as antigen presentation and cancer immunotherapy (40). Hemoglobin transports oxygen for all organs of the body and some research demonstrated that HGB can interact with the innate immune system either directly or through binding to pathogen-associated molecular patterns (41). Platelets are closely related to the inflammatory process of many types of diseases and exhibit complex interactions with their circulating cells and blood vessel walls (42). In this experiment, the level of WBC, LYM, RBC, HGB, and PLT decreased by the injection of CTX. However, those levels in mice administrated with ACP got enhanced significantly compared with the model group. This indicated that ACP could improve the decline of RBC, WBC, Hb, and PLT levels induced by CTX and restore mouse immunity.

As the intercellular signaling peptides, cytokines are especially important in the immune system. In the body’s immune response, it can promote the proliferation and differentiation of target cells, induce receptor expression, and enhance the body’s resistance to infection, thus playing an important role in regulating the body’s immune and inflammatory response. TNF-α, produced mainly by activated macrophages, plays different roles in regulating multiple developmental and immune processes, such as killing and inhibiting tumor cells (43). IFN-γ is an important immune response molecule, which can activate macrophages and produce inducible nitric oxide synthase (iNOS), to promote NO synthesis. IFN-γ is the first line of defense against antiviral infection, especially it plays a crucial role in cellular immunity (44). As a key multifunctional cytokine, IL-1β is involved in both autoimmune inflammatory responses.
and a range of cellular activities, such as cell proliferation, differentiation, and apoptosis (45, 46). Produced by activated monocytes, fibroblasts, endothelial cells, and so on, IL-6 can stimulate lymphocytes proliferation and promote the production of immunoglobulin by B cells (47). Previous studies have shown that some polysaccharides can activate immune cells and increase cytokines and immunoglobulin levels, thereby alleviating CTX-induced immune damage. *Dendrobium sonia* polysaccharides (DSP) can significantly increase the secretion of TNF-α, IL-6, and IFN-γ in serum, thus showing good immune-enhancing ability (48). *Millettia speciosa* Champ polysaccharides can improve CTX-induced immunosuppression by upregulating serum levels of IL-2, IL-4, IL-10, TNF-α, and IgG (49). Immunoglobulin is a kind of protein that is used to protect against a variety of pathogens, including bacteria, viruses, cancer cells, and other harmful substances, which is mainly secreted by plasmacyte (50).

IgM is the immunoglobulin with the largest molecular weight, which is mainly secreted and in humoral immune response first, accounting for 5–10% of the total immunoglobulin in serum. IgM has powerful functions of sterilization, complement activation, immune conditioning, and agglutination (51). IgG is the most important antibody in the body, accounting for approximately 75% of the total immunoglobulin content in the serum. It has the functions of a neutralizing virus, antibacterial, and immune regulation, and plays an important role in neonatal anti-infection (52). The decline in the expression of TNF-α, IFN-γ, IL-1β, IL-6, Ig-M, and Ig-G in serum of the CTX model group was found in our study, which means the immune system of mice got damaged by the injection of CTX. It was observed that there was a similar trend in the production of cytokines and immunoglobulins among the groups. The results showed that ACP can promote the secretion of TNF-α, IFN-γ, IL-1β, IL-6,
FIGURE 6 | Effect of ACP_{60}, ACP_{80}, and ACP_{t2} on liver T-SOD (A), GSH-Px (B), and MDA (C) levels in CTX-mice. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01 vs. the normal group. #P < 0.05, ##P < 0.01 vs. the model group.
REFERENCES

1. Nanjing Univ Chinese Med. Traditional Chinese Medicine Dictionary. 1st ed. Shanghai: Shanghai Science & Technology Press (2014).
2. Yang M, Al Zaharna M, Chen Y, Li L, Cheung H. Phytochemical profiles, antioxidant activities of functional herb Abrus cantoniensis. Int J Biol Macromol. (2016) 81:784–90. doi: 10.1016/j.ijbiomac.2016.04.163
3. Li H, Song ZJ Dai YP, Zhang SL, He X, Guo CR, et al. Antioxidative activity of flavonoids from Abrus cantoniensis against ethanol-induced gastric ulcer in mice. Planta Med. (2015) 81:784–90. doi: 10.1055/s-0035-1546080
4. Li D, Zheng X, Duan L, Deng S, Ye W, Wang A, et al. Ethnobotanical survey of herbal tea plants from the traditional markets in Chaoxian, China. J Ethnopharmacol. (2017) 205:195–206. doi: 10.1016/j.jep.2017.02.040
5. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People’s Republic of China. Part I. Beijing: China Medical Science and Technology Press (2020).
6. Zeng Q, Xie H, Song H, Nie F, Wang J, Chen D, et al. In vivo wound healing activity of Abrus cantoniensis extract. Evid-Based Compl Alt. (2016) 2016:1–7. doi: 10.1155/2016/6568528
7. Yang M, Shen Q, Li L, Huang Y, Cheung H. Phytochemical profiles, antioxidant activities of functional herb Abrus cantoniensis and Abrus mollis. Food Chem. (2015) 177:304–12. doi: 10.1016/j.foodchem.2015.01.054
8. Zhu Y, Yu X, Ge Q, Li J, Wang D, Wei Y, et al. Antioxidant and anti-aging activities of polysaccharides from Cordyceps cicadae. Int J Biol Macromol. (2020) 157:394–400. doi: 10.1016/j.ijbiomac.2020.04.163
9. Zhang S, Zhang Q, An L, Zhang J, Li Z, Zhang J, et al. A fructan from Anemarrhena asphodeloides Bunge showing...
neuroprotective and immunoregulatory effects. *Carbohydr. Polym.* (2020) 229:115477. doi: 10.1016/j.carbpol.2019.115477

10. Guo WL, Chen M, Pan WL, Zhang Q, Xu JX, Lin YC, et al. Hypoglycemic and hypolipidemic mechanism of organic chromium derived from chelation of *Grifola frondosa* polysaccharide-chromium (III) and its modulation of intestinal microflora in high fat-diet and STZ-diabetic mice. *Int J Biol Macromol.* (2020) 145:1208–18. doi: 10.1016/j.ijbiomac.2019.09.206

11. Zhang Y, Zeng Y, Cui Y, Liu H, Dong G, Sun Y. Structural characterization, antioxidiant and immunomodulatory activities of a novel polysaccharide from *Cordyceps militaris* cultivated on hull-less barley. *Carbohydr. Polym.* (2020) 235:115969. doi: 10.1016/j.carbpol.2020.115969

12. Cao M, Wang S, Gao Y, Pan X, Wang X, Deng R, et al. Study on physicochemical properties and antioxidant activity of polysaccharides from *Desmodium armatum*. *J Food Biochem.* (2020) 4/e13243. doi: 10.1111/jfbc.13243

13. Yoo HJ, You D, Lee K. Characterization and immunomodulatory effects of high molecular weight fucoidan fraction from the Sporophyll of *Undaria pinnatifida* in cyclophosphamide-induced immunosuppressed mice. *Mar Drugs.* (2019) 17:447. doi: 10.3390/md17080447

14. Zhou X, Dong Q, Kan X, Peng L, Xu X, Fang Y, et al. Immunomodulatory activity of a novel polysaccharide from *Lonicera japonica* in immunosuppressed mice induced by cyclophosphamide. *PLoS ONE.* (2018) 13:e020152. doi: 10.1371/journal.pone.020152

15. Chen L, Qi Y, Qi Z, Gao K, Gong R, Shao Z, et al. A comparative study on the effects of different parts of *Panax ginseng* on the immune activity of cyclophosphamide-induced immunosuppressed mice. *Molecules.* (2019) 24:1096. doi: 10.3390/molecules24061096

16. Cui HY, Wang CL, Wang YR Li ZJ, Chen MH Li FJ, et al. A polysaccharide from *Pleurotus nebrodensis* and hypolipidemic mechanism of organic chromium derived from chelation of *Acanthosyce canthinicus* by response surface methodology and evaluation of its anti-inflammatory effect. *Molecules.* (2022) 27:2036. doi: 10.3390/molecules2702036

17. Li W, Li L, Zhen W, Wang L, Pan M, Lv J, et al. Process Biochem. (2020) 10:419. doi: 10.3390/polym10040419

18. Wang Q, Meng X, Zhu L, Xu Y, Cui W, He X, et al. A polysaccharide found in *Psalotoma fortunei* flowers can enhance cellular and humoral immunity in chicken. *Int J Biol Macromol.* (2019) 130:213–9. doi: 10.1016/j.ijbiomac.2019.01.168

19. Zhou W, Zhao Y, Yan Y, Mi J, Lu L, Luo Q, et al. Antioxidant and immunomodulatory activities in vitro of polysaccharides from bee collected pollen of Chinese wolfberry. *Int J Biol Macromol.* (2020) 163:190–9. doi: 10.1016/j.ijbiomac.2020.06.244

20. Wang L, Li L, Gao J, Huang J, Yang Y, Xu Y, et al. Characterization, antioxidiant and immunomodulatory effects of selenized polysaccharides from dandelion roots. *Carbohydr. Polym.* (2021) 266:117796. doi: 10.1016/j.carbpol.2021.117796

21. Wu E, Sun W, Wang Y, Zhang G, Xu B, Chen X, et al. Optimization of ultrasonic-assisted extraction of total flavonoids from *Astrum cantoniensis* (Abriherba) by response surface methodology and evaluation of its anti-inflammatory effect. *Molecules.* (2022) 10.3390/molecules2702036

22. Chen X, Cai B, Wang J, Sheng Z, Yang H, Wang D, et al. Mulberry leaf-derived polysaccharide modulates the immune response and gut microbiota composition in immunosuppressed mice. *J Funct. Foods.* (2021) 83:105454. doi: 10.1016/j.jff.2021.105454

23. Wu F, Huang H. Surface morphology and protective effect of *Heritum Erinaceus* polysaccharide on cyclophosphamide-induced immunosupression in mice. *Carbohydr. Polym.* (2020) 251:116930. doi: 10.1016/j.carbpol.2020.116930

24. Dai J, Chen J, Qi J, Ding M, Liu W, Shao T, et al. Konjac glucomannan from *Amorphophallus konjac* enhances immunocompetence of the cyclophosphamide-induced immunosuppressed mice. *Food Sci Nutr.* (2021) 9:728–35. doi: 10.1002/fsn3.2038

25. Li Q, Zhang F, Chen G, Chen Y, Zhang W, Mao G, et al. Purification, characterization and immunomodulatory activity of a novel polysaccharide from *Grifola frondosa*. *Int J Biol Macromol.* (2018) 111:1293–303. doi: 10.1016/j.ijbiomac.2018.01.090

26. Dispenza MC. Classification of hypersensitivity reactions. *Allergy Asthma Proc.* (2019) 40:470–3. doi: 10.2500/aap.2019.40.4274

27. Otani IM, Banerji A. Immediate and delayed hypersensitivity reactions to corticosteroids: evaluation and management. *Curr Allergy Asthm R.* (2016) 16:18. doi: 10.1007/s11882-016-0596-7

28. Yuandani, Nugraha SE, Laila I, Satria D. Immunomodulatory effects of standardized extract of *Curcuma mangga* val. On cytokines, antibody and delayed-type hypersensitivity response in Wistar rats. *Res Pharm Sci.* (2021) 16:16–25. doi: 10.4103/1735-5362.305185

29. Pauling K, Schwenck J, Lemberg C, Trautwein C, Zissemare L, Kramer D, et al. Immunomodulatory role of reactive oxygen species and nitrogen species during T cell-driven neutrophil-enchaced acute and chronic cutaneous delayed-type hypersensitivity reactions. *Theranostics.* (2021) 11:470–90. doi: 10.7150/thno.51462

30. Sun W, Meng K, Qi C, Yang X, Wang Y, Fan W, et al. Immune-enhancing activity of polysaccharides isolated from *Atractylodis macrocephalae* Koidz. *Carbohydr. Polym.* (2015) 126:91–6. doi: 10.1016/j.carbpol.2015.03.034

31. Crounkt DA, Strutt TM. The regulation of inflammation by innate and adaptive lymphocytes. *J Immunol Res.* (2018) 2018:14. doi: 10.1155/2018/1467588

32. Sun X, Han X, Xu L, Gao M, Xu J, Yang R, et al. Surface-engineering of red blood cells as artificial antigen presenting cells promising for cancer immunotherapy. *Small.* (2017) 13:1701864. doi: 10.1002/sml2.201701864

33. Lee SK, Ding JL. A perspective on the role of extracellular hemoglobin on the innate immune system. *DNA Cell Biol.* (2013) 32:36–40. doi: 10.1089/dna.2012.1897

34. Koupnova M, Clancy L, Corkrey HA, Freedman J.E. Circulating platelets as mediators of immunity, inflammation, and thrombosis. *Circ Res.* (2018) 122:337–51. doi: 10.1161/CIRCRESAHA.117.310795

35. Huo J, Wu J, Zhao M, Sun W, Sun L, Ji H, et al. Immunomodulatory activity of a novel polysaccharide extracted from Huangshui on THP-1 cells through NO production and increased IL-6 and TNF-α expression. *Food Chem.* (2020) 330:127257. doi: 10.1016/j.foodchem.2020.127257

36. Wang H, Xu L, Yu M, Wang Y, Jiang T, Yang S, et al. Glycosaminoglycan from *Apostichopus japonicus* induces immunomodulatory activity in cyclophosphamide-treated mice and in macrophages. *Int J Biol Macromol.* (2019) 130:229–37. doi: 10.1016/j.ijbiomac.2019.02.093

37. Sun W, Hu W, Meng K, Yang L, Zhang W, Song X, et al. Activation of macrophages by the ophiopogon polysaccharide liposome from the root tuber of *Ophiopogon japonicus*. *Int J Biol Macromol.* (2016) 91:918–25. doi: 10.1016/j.ijbiomac.2016.06.037

38. Gao X, Qi J, Ho C, Li B, Mu J, Zhang Y, et al. Structural characterization and immunomodulatory activity of a water-soluble polysaccharide from *Ganoderma lucidum* fruiting bodies.
47. Dong Z, Zhang M, Li H, Zhan Q, Lai F, Wu H. Structural characterization and immunomodulatory activity of a novel polysaccharide from *Pueraria lobata* (Wild) Ohwi root. *Int J Biol Macromol.* (2020) 154:1556–64. doi: 10.1016/j.ijbiomac.2019.11.040

48. Liu W, Yan R, Zhang L. Dendrobium sonia polysaccharide regulates immunity and restores the dysbiosis of the gut microbiota of the cyclophosphamide-induced immunosuppressed mice. *Chin J Nat Med.* (2019) 17:600–7. doi: 10.1016/S1875-5364(19)30062-7

49. Chen X, Sun W, Xu B, Wu E, Cui Y, Hao K, et al. Polysaccharides from the roots of millettia speciosa champ modulate gut health and ameliorate cyclophosphamide-induced intestinal injury and immunosuppression. *Front Immunol.* (2021) 12:766296. doi: 10.3389/fimmu.2021.766296

50. Turula H, Wobus C. The role of the polymeric immunoglobulin receptor and secretory immunoglobulins during mucosal infection and immunity. *Viruses.* (2018) 10:237. doi: 10.3390/v10050237

51. Kao D, Lux A, Schaffert A, Lang R, Altmann F, Nimmerjahn F. Natural autoantibodies in physiology and the treatment of disease. *Methods Mol Biol.* (2019) 1904:53–81. doi: 10.1007/978-1-4939-8958-4_3

52. Kao D, Lux A, Schaffert A, Lang R, Altmann F, Nimmerjahn F. IgG subclass and vaccination stimulus determine changes in antigen specific antibody glycosylation in mice. *Eur J Immunol.* (2017) 47:2070–9. doi: 10.1002/eji.201747208

53. Loukotova L, Konefal R, Venclova K, Machova D, Janouskova O, Rabky M, et al. Hybrid thermoresponsive graft constructs of fungal polysaccharide beta-glucan: physico-chemical and immunomodulatory properties. *Eur Polym J.* (2018) 106:118–27. doi: 10.1016/j.eurpolymj.2018.07.004

54. Nwanna EE, Oyelaye SI, Oboh G. Editan (*Lasianthera africana*) leaf-inclusive diets modulate some neuronal enzyme activities and antioxidant status of cyclophosphamide-treated Wistar rats. *J Food Biochem.* (2021) 45:e13427. doi: 10.1111/jfbc.13427

55. Ghosh A, Rabbani SI, Asdaq SMB, Mohzari Y, Alrashed A, Najib Alajami H, et al. *Morus alba* prevented the cyclophosphamide induced somatic and germinal cell damage in male rats by ameliorating the antioxidant enzyme levels. *Molecules.* (2021) 26:1266. doi: 10.3390/molecules26051266

56. Wang M, Meng XY, Yang RL, Qin T, Wang XY, Zhang KY, et al. *Cordyceps militaris* polysaccharides can enhance the immunity and antioxidation activity in immunosuppressed mice. *Carbohydr Polym.* (2012) 89:461–6. doi: 10.1016/j.carbpol.2012.03.029

57. Xie Y, Wang L, Sun H, Wang Y, Yang Z, Zhang G, et al. Immunomodulatory, antioxidant and intestinal morphology-regulating activities of alfalfa polysaccharides in mice. *Int J Biol Macromol.* (2019) 133:1107–14. doi: 10.1016/j.ijbiomac.2019.04.144

58. Chen S, Wang J, Fang Q, Dong N, Nie S. Polysaccharide from natural *Cordyceps sinensis* ameliorated intestinal injury and enhanced antioxidant activity in immunosuppressed mice. *Food Hydrocolloid.* (2019) 89:661–7. doi: 10.1016/j.foodhyd.2018.11.018

59. Ye X, An Q, Chen S, Liu X, Wang N, Li X, et al. The structural characteristics, antioxidant and hepatoprotective activities of polysaccharides from *Chimonanthus nitens* Oliv. *Leaves Int J Biol Macromol.* (2020) 156:1520–9. doi: 10.1016/j.ijbiomac.2019.11.200

60. Zhang Y, Han Y, He J, Ouyang K, Zhao M, Cai L, et al. Digestive properties and effects of *Chimonanthus nitens* Oliv polysaccharides on antioxidant effects in vitro and in immunocompromised mice. *Int J Biol Macromol.* (2021) 185:306–16. doi: 10.1016/j.ijbiomac.2021.06.114

61. Shang X, Chao Y, Zhang Y, Lu C, Xu C, Niu W. Immunomodulatory and antioxidant effects of polysaccharides from *Gynostemma pentaphyllum* Makino in immunosuppressed mice. *Molecules.* (2016) 21:1085. doi: 10.3390/molecules21061085

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Qu, Hu, Lian, Sun and Si. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.