Regulation of cyclophosphamide-induced immune activity in mice by arabinogalactan

Kening Xie[a], Mingguo Sun[b], Shang Qingman Xin[a], Wei Zhang[a], Shengxue Zhou[a]*
[a]Traditional Chinese Medicine Department, Jilin Agricultural Science and Technology University, No.77 Hanlin Road, Jiuzhan Street, Changyi District, Jilin, 132101, China; [b]Linjiang Special Industry Development Service Center, Linjiang, 134600, China
*zhoushengxue1111@163. com

ABSTRACT

Objective: The effect of arabinogalactan on immune function in mice has been systematically studied to provide experimental and theoretical basis for clinical and food development and application. **Method:** Kunming mice were randomly divided into four groups, and a mouse immunosuppressive model was established using cyclophosphamide as a tool drug. Blood routine, immune organs, liver cell physiological sections, and antigen physiological indicators (AST, ALT, MDA, TNF-α and IL-β) in the blood were measured. In vitro lymphocyte proliferation and transformation were also evaluated. **Result:** We successfully established a cyclophosphamide-induced immunosuppressive mouse model. In the arabinogalactan-treated and the cyclophosphamide-treated groups, increases were observed in body weight, thymus and spleen weight, red blood cells, white blood cells, and lymphocytes. The results of liver tissue sectioning and physiological index analysis showed that the arabinogalactan group could positively regulate various indicators compared to the control group, with significant differences; The positive regulation of arabinogalactan and cyclophosphamide groups was significant compared to the cyclophosphamide group, and the difference was extremely significant; The single use of arabinogalactan has the best effect on various indicators in mice. **Conclusion:** Arabinogalactan has an antagonistic effect on immunosuppressants, improves various indicators of mouse immune function, and has the effect of enhancing mouse immune function.

Keywords: Arabinogalactan, immunization, cyclophosphamide

1. INTRODUCION

Arabinogalactan (AG) is composed of arabinose and galactan [1], which has a long branched macromolecular structure and is a water-soluble neutral polysaccharide. This kind of polysaccharide is mainly enriched in the xylem of conifers, especially in Larch (Larix olgensis) up to 5~30% [2,3]. AG is similar to Arabic gum in nature [4]. A large number of studies have verified that AG is not digested and absorbed by the human body in the stomach and small intestine, but can be degraded by microorganisms in the large intestine [5]. AG is often used as thickener, stabilizer, emulsifier, sweetener, etc. in the food industry. It can also be used as the base material of seasoning, emulsified essence, pudding and sauce, etc. it can improve the taste in food. One of the characteristics is used in bread, sausage and noodles. In the toxicological test in mice, eating AG is not toxic and will not produce side effects [6]. Foreign scholars have done a lot of pharmacological research on AG, which has the activity of stimulating the immune system [7], preventing colds caused by its immune stimulating properties [8], promoting the reproduction and growth of beneficial bacteria in the intestine [9] and other physiological functions. The aim of this study was to investigate the effect of AG on immune function and blood routine in mice, and to establish the Immunosuppressive Model of mice with cyclophosphamide (CP) drug, which provided experimental basis and theoretical basis for clinical and food development and application.

2. MATERIALS AND METHODS

2.1 Materials and instrument

Arabinogalactan, provided by Changchun Zhongxing Hengyuan Pharmaceutical Technology Co., Ltd., is extracted and purified from Larix olgensis with a purity of more than 90%. Kunming mice, ♀♂ All use, (Changchun medical animal experimental research center). Cyclophosphamide tablets were purchased from Tonghua Maoxiang Pharmaceutical Co., Ltd. (h22026738), concanavalin A (ConA, sigma), lipopolysaccharide (LPS, sigma), diphenyltetrazolium bromide (MTT,

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sigma), dimethyl sulfoxide (DMSO, Beijing chemical plant), calf serum (Hangzhou Sijiqing biological engineering materials Co., Ltd.), hematoxylin and eosin (h&e) dyes, AST, alt, MDA, TNF - α and il- β analysis kits (Nanjing Jiancheng Biotechnology Institute). Centrifuge (Eppenderf, 5430R), Microplate reader (Spectramax plus-384, Meigu molecular instrument Co., Ltd.), High-speed centrifuge (TG16G-IV, Hunan Kaida Scientific Instrument Co., Ltd.), Incubator (Thermo Co., Ltd, USA).

2.2 Effect of AG on growth and blood routine of mice

Forty mice weighing 18-20g were randomly divided into four groups: control group (5% normal saline 100mg · kg-1); Cyclophophamide (CP) Group (100mg · kg-1); AG group (100 mg · kg-1); CP+AG (100mg · kg-1) group. The drug was administered by gavage daily at the specified dose for 14 consecutive days. 24 hours after the last administration, blood samples were taken from the eyeballs to measure blood routine, and edtk-2 anticoagulant tube was injected. Experimental references [10].

2.3 Effect of AG on immune organs of mice

After the completion of blood collection, the mice were dislocated and killed. The weights of thymus and spleen of mice were accurately measured under sterile conditions. The ratio of the weight of thymus and spleen (mg) to body weight (g) was used as the index of thymus and spleen. The spleen was washed twice with Hanks' solution, cut into pieces, ground to make spleen cell suspension, and the spleen was filtered with a 200 mesh net to form a 2 × 106 cell suspension. Count under the microscope and convert it to the total number of cells in each organ [10].

2.4 Determination of inflammatory factors in serum

The cervical dislocation of mice was dissected, blood was collected from the eyeball, centrifuged at 3000rpm for 10 minutes, and the bleeding serum was separated from the supernatant and stored at − 80 ℃. Then the contents of TNF - α and IL-1 ß in the serum of mice were detected, the OD value was determined and the content was calculated, and the operation was carried out according to the steps in the manual.

2.5 H&E staining

In order to study the liver tissue changes of hepatocyte necrosis, apoptosis and central venous congestion, fresh liver samples were soaked in 10% formaldehyde for more than 24 hours. Then the liver tissue was embedded in paraffin and made into sections. Next, 5 μ m thick sections were stained with h&e under optical microscope. Image pro and 6.0 software were used to evaluate the degree of liver apoptosis by quantitative fragmentation and concentrated staining.

2.6 Llymph node proliferation test in vitro

BALB/c mice were killed by dislocation and prepared into 2 × 106 spleen cell suspension for use. The method is the same as that in 1.3.2. The experiment was divided into blank group, negative control group, positive control group and sample group (5% normal saline, CP Group, AG group, CP+AG group 10 μ g · ML-1). The adjusted cell suspension was added to the 96 well cell culture plate, the blank was added with 100 μ l culture medium, the other groups were added with 100 μ L cell suspension, cultured at 37 ℃ for 1h, the blank was added with 100 μ l culture medium, the positive control was added with 50 μ G/ml ConA 100 μ L, the sample group was added with 100 μ l solution of each group, cultured at 37 ℃, 5% CO2 incubator for 48h, each well was added with 5mg/ml MTT 20 μ L for 4h, centrifuged at 2000r/min for 10min, went to the clear night, added with 150 μ l DMSO to fully crystallize, and the OD value was measured at 570 nm by microplate reader.

2.7 Lymphocyte transformation in vivo

After 14 days of oral administration, the spleen of mice in each group was taken and made into cell suspension 2 × 106. Each group was divided into three groups: the first group was the natural transformation of spleen lymphocytes; In the second group, ConA was added to stimulate T cell transformation; In the third group, LPS was added to stimulate the transformation of B cells. The culture method is the same as that in 2.3.

2.8 Analysis of the composition of the intestinal flora of mice

After 14 days of CP induced, the feces of each group of mice were collected, placed in a 10 mL dry sterilized centrifuge tube, and stored in a refrigerator at -80 °C for testing. The QIAamp DNA Stool Mini Kit (QIAGEN, Germany) was used to extract bacterial genomic DNA, and all operations were performed in strict accordance with the requirements of the

kit. The extracted genomic DNA is stored at -20 ℃ after concentration and purity determination. The fecal intestinal flora composition was analyzed by ARISA (automated ribosomal intergenic-spacer analysis), and PCR amplification primers were ITS-F (5'-GTCGTAACAAGGTAGCCGTA-3') and ITS-R (5'- GCCAAGGCATCCAC-3'). Take 5 μL of PCR amplification product for community composition analysis (ABIPRISM 3100 Genetic Analyzer). The sequencing data was compared through the bioinformation cloud i-singer platform, the species abundance of each sample was counted at different classification levels, and the composition of the intestinal bacterial community was analyzed.

2.9 Statistical treatment

SPSS17.0 software was used, the data were expressed in (x ± s), and the statistical method was single factor analysis.

3. RESULTS AND ANALYSIS

3.1 Blood routine analysis of mice

The mice in the arabinogalactan (AG) group were stronger than those in the control group (CK group) and cyclophosphamide (CP) group, with smooth and bright hair, very active waves, and increased food intake. The activity of the mice in the arabinogalactan and cyclophosphamide (AG+CP) group was stronger than that in the blank control group and cyclophosphamide group, with neat hair color and increased food intake. The blood routine and hemoglobin test results are shown in Table 1.

Table 1. Effect of arabinogalactan (AG) on blood routine and hemoglobin of mice n=10 (x ± s)

Group	RBC ($10^{12}\cdot L^{-1}$)	Hb ($g\cdot L^{-1}$)	WBC ($10^{9}\cdot L^{-1}$)	LC %	Neutrophil %	Monocyte %
CK	6.68±0.44	124±13	1.18±0.34	62.3±7.4	35±8.4	0.9±0.3
CP	4.35±0.43^{a}	86±14^{a}	0.7±0.34^{a}	45.6±7.2^{a}	28±6.4^{b}	0.6±0.5^{b}
AG	7.39±0.46^{b}	135±15^{c}	1.74±0.41^{bc}	74.1±7.9^{b}	36±8.6	0.8±0.4
AG+CP	7.11±0.47^{c}	130±12^{c}	1.54±0.37^{c}	71.5±6.8^{d}	33±7.2^{c}	0.7±0.3^{c}

a: p<0.01, b: p<0.05 to CK group; c: p<0.01, d: p<0.05 to CP group.

It can be seen from table 1 that the number of red blood cells, white blood cells and lymphocytes increased in the arabinogalactan (AG) group and the arabinogalactan and cyclophosphamide (AG+CP) group. There was significant difference between AG and CK, and there was significant difference between AG+CP and CP. There were no significant differences in hemoglobin, neutrophils and monocytes between AG+CP group and control group, but there were significant differences between AG+CP Group and CP Group.

3.2 Effect of arabinogalactan (AG) on the weight of immune organs in mice

Table 2. Effect of arabinogalactan (AG) on the weight of immune organs in mice n=10 (x ± s)

Group	Weight/g	Thymus weight/mg	Thymus index/($mg.g^{-1}$)	Spleen weight/mg	Spleen index/($mg.g^{-1}$)
CK	23.86±0.73	76.83	3.22±0.11	114.77	4.87±0.16
CP	22.35±0.43	65.71	2.94±0.16^{a}	94.09	4.21±0.23^{b}
AG	25.32±0.95	119.26	4.71±0.13^{a}	163.82	6.74±0.17^{a}
AG+CP	24.55±0.67	100.90	4.11±0.15^{c}	137.73	5.61±0.08^{c}

a: p<0.01, b: p<0.05 to CK group; c: p<0.01, d: p<0.05 to CP group.

It can be seen from table 2 that the body weight, thymus and spleen weight of mice taking cyclophosphamide orally decreased, which was lower than that of the control group. The body weight, thymus and spleen weight of AG group and AG+CP group were significantly higher than those of CK and CP Group; Compared with the thymus and spleen index, the AG group was significantly higher CK group, and the AG+CP Group was higher than CP Group. These results

indicate that arabinogalactan has a positive effect on the body weight, thymus and spleen weight of mice. Arabinogalactan can regulate the immunosuppression of cyclophosphamide and improve the recovery of the body.

3.3 Effect of arabinogalactan (AG) on the number of cells in immune organs of mice

According to Table 3, the number of cells and lymph node cells in AG group and AG+CP group were significantly higher than those in CP Group ($p<0.01$). Compared with CK group, the spleen cells of AG+CP group were lower and lymphocytes were higher than those of control group, the differences were not significant. The number of spleen cells and lymph node cells in CP Group was the lowest, which showed that arabinogalactan could significantly increase the number of spleen cells and lymphocytes in mice, and had an obvious antagonistic effect on the decrease of the number of cells caused by cyclophosphamide.

Table 3. Effect of arabinogalactan (AG) on the number of cells in immune organs of mice n=10 ($x \pm s$)

Group	Number of spleen cells ($\times 10^6$)	Number of lymph node cells ($\times 10^6$)
CK	0.58±0.07	0.30±0.05
CP	0.26±0.04[a]	0.19±0.04[a]
AG	0.67±0.12[b]	0.38±0.05[b]
AG+CP	0.51±0.10[c]	0.33±0.03[c]

a: $p<0.01$, b: $p<0.05$ to CK group; c: $p<0.01$, d: $p<0.05$ to CP group.

3.4 Effect of arabinogalactan (AG) on liver of mice

As shown in Figure 1, it can be seen from the picture analysis of mouse liver tissue induced by CP that the cavitation of hepatocytes is very obvious in the whole microscopic field of vision, and a large number of cell death phenomena have occurred. However, the hepatocytes in the CK and AG groups had compact structure, little cavitation, clear cell contour, obvious hepatic lobules, and no histopathological changes. In the CP group treated with AG, hepatocytes showed partial cavitation, but it was significantly better than that in the CP Group and played a regulatory role (Fig. 1).

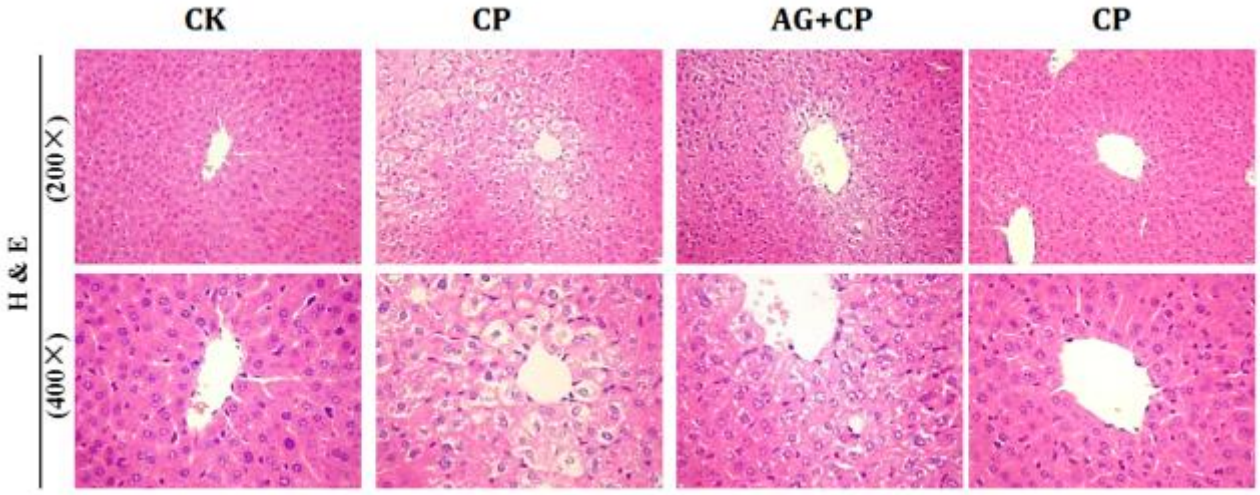

Figure 1. Liver tissue was stained with hematoxylin eosin (H & E) (200 ×, 400 ×)

3.5 Determination of physiological indexes of arabinogalactan (AG) in mice

The contents of AST, MDA, TNF-α and IL-β in CP model group were significantly higher than those in the control group ($p<0.01$). The contents of four substances in AG group and AG+CP group were significantly lower than those in

the control group (P<0.05). The content of ALT showed an opposite trend, and the content of ALT in AG and AG+CP groups showed an upward trend, which was significantly different from that in CP Group (p<0.01). The experimental data showed that the contents of AST, MDA, TNF - α and IL - β in mice treated with AG were significantly decreased, and the content of ALT was increased. It can be concluded that AG has a tendency to improve the immune function of mice induced by CP (Table 4).

Table 4. Determination of physiological indexes of arabinogalactan (AG) in mice

	CK	CP	AG+CP	AG
ALT(U/L.protein)	48.87±3.21	19.64±1.56 [a]	43.56±2.88[c]	55.23±3.24[c]
AST(U/L.protein)	43.52±1.45	275.9±13.12[a]	165.45±3.21[c]	41.87±3.45[c]
MDA (μmol/mg.protein)	3.86±0.23	6.34±1.36[a]	4.83±0.31[c]	3.98±0.36[c]
TNF-α(pg/mg.protein)	176.32±20.31	386.54±35.08[a]	301.5±28.12[c]	180.56±17.23[c]
IL-β(pg/mg.protein)	135.3±12.15	396.7±40.12[a]	300.25±30.14[c]	153.4±15.26[c]

a: p<0.01, b: p<0.05 to CK group; c: p<0.01, d: p<0.05 to CP group.

3.6 In vitro lymphocyte proliferation of arabinogalactan (AG)

Figure2 shows that the in vitro proliferation test showed that the proliferation of lymphocytes in the AG group was significantly higher than that in the CK (87%) group, and the AG+CP (67%) group was significantly better than that in the CP (25%) group, and the difference was extremely significant (p<0.01). The proliferation rate in vitro in the AG group was the highest in the four groups, indicating that arabinogalactan can proliferate lymphocytes and offset the damage of cyclophosphamide to lymphocytes.

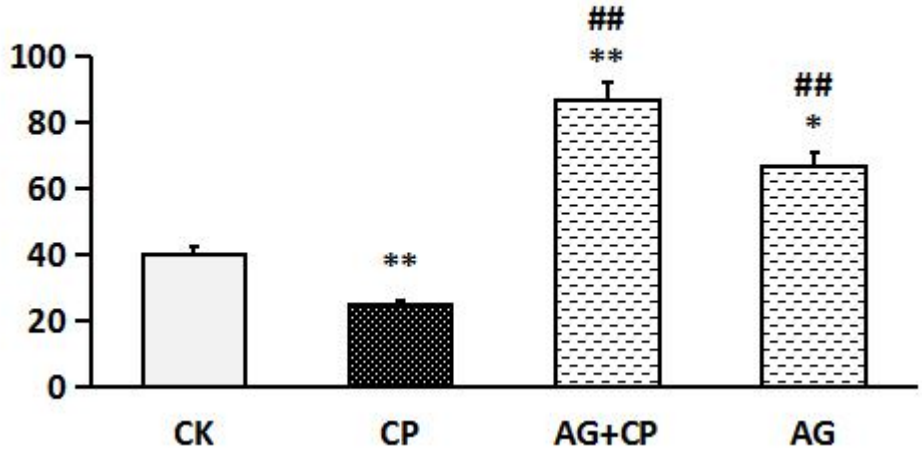

Figure 2. Lymphocyte proliferation rate in vitro (*p < 0.05, **p < 0.01 to CK group; #p < 0.05, ##p < 0.01 to CP group)

3.7 Effect of arabinogalactan (AG) on lymphocytes in mice

After 14 days of intragastric administration, the spleen was taken for treatment, and T, B lymphocyte transformation was carried out. T lymphocyte transformation. With ConA as a stimulant, T lymphocyte transformation was significantly increased in AG group and AG+CP group (Fig. 3a), while for B lymphocyte transformation, the increase in AG group was significant, and the increase in AG+CP group was not significant compared with CK (Fig. 3b), but for CP Group, both had a significant increase, and the difference was extremely significant. It can be seen that arabinogalactan can increase the proliferation of lymphocytes (350% for T lymphocytes and 186% for B lymphocytes), and can also inhibit the destruction of cyclophosphamide on the body's immunity and improve immunity.

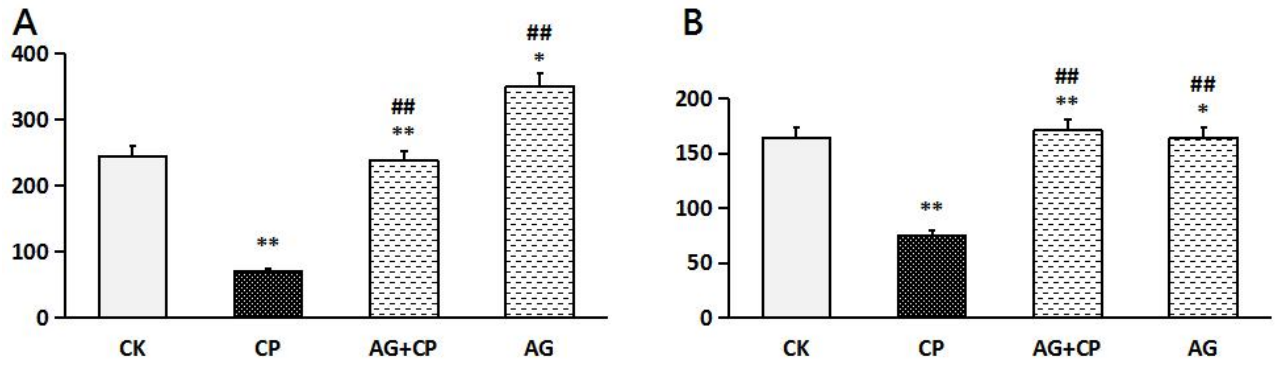

Figure 3. Lymphocyte transformation rate in vivo. A. T lymphocyte transformation rate; B. B lymphocyte transformation rate. (*p < 0.05, **p < 0.01 to CK group; #p < 0.05, ##p < 0.01 to CP group)

3.8 AG Induced Structural Changes in Gut Microbiota in mice

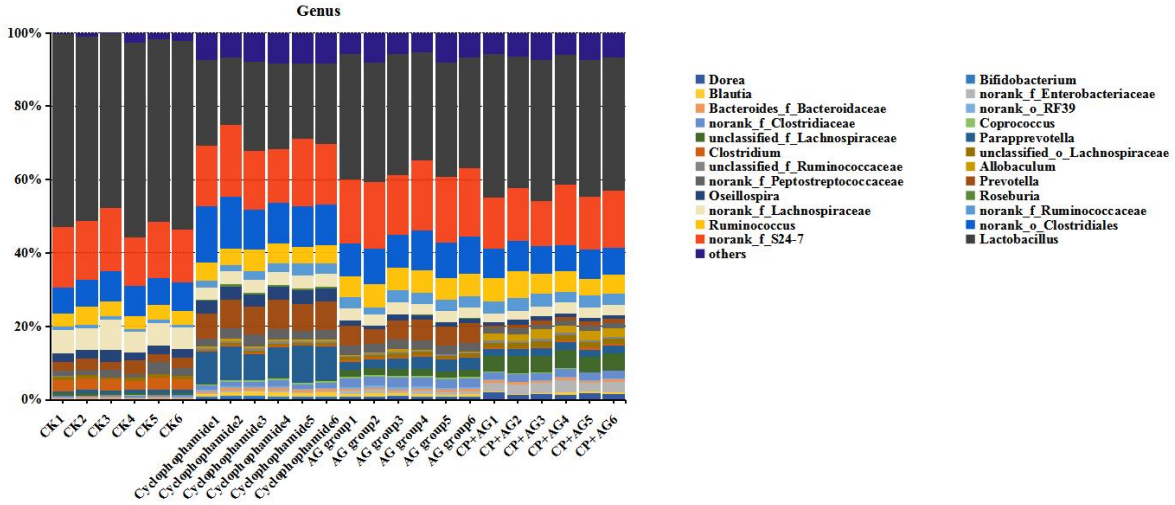

Figure 4 .AG treatment regulated gut microbiota structure in APAP induced liver injury. Relative abundances of gut microbiota at the genus level. (n=6).

At the genus level (Figure 4), the intestinal flora of each group of mice is mainly composed of Lactobacillus, norank_f_S24_7, norank_o_Clostridiales, Ruminococcus, norank_f_Ruminococcaceae, norank_f_Lachnospiraceae, Oscillospira and Prevotella. The abundance of Lactobacillus in the intestine of the CP-induced model group was significantly lower than that of the normal group, and the relative abundance of norank_o_Clostridiales, norank_f_Ruminococcaceae, Oscillospira, and Prevotella increased significantly. Compared with the CP induction group, the proportion of Lactobacillus in the intestine of mice in the POP and POP+CP groups increased significantly, and the relative proportions of norank_o_Clostridiales and Prevotella decreased significantly.

4. DISCUSSION

In this experiment, the immunosuppressive mouse model was successfully established by cyclophosphamide on the basis of predecessors. The spleen cells and lymphocytes were significantly reduced, and the morphology and blood routine were significantly lower than those of the blank control group. Microscopic observation of the liver also verified that AG could adjust the immune response. By measuring the contents of AST, MDA, alt, TNF - α and IL - β, arabinogalactan could regulate the expression of related contents, protect the body and regulate immunity. The proliferation rate in vitro was lower than that of the control, and the T lymphocytes and B lymphocytes in vivo were lower than those of the blank control. Arabinogalactan can significantly resist the immunosuppressive effect of cyclophosphamide, and adjust the

indicators of immunosuppressive mice to reach or even higher than the control group. Arabinogalactan alone significantly increased the body weight, thymus and spleen weight, blood routine value, spleen, alt, lymphocyte number and lymphocytic t (350%) and B (186%) cell transformation rate of mice, reduced liver injury, and reduced the contents of AST, MDA, TNF - α and IL - β, which played a positive regulating role. AG is a water-soluble polysaccharide that cannot be absorbed by the human body. Its main function is to regulate the balance of intestinal microbiota, protect intestinal mucosa, and then improve liver damage and enhance the immune system through intestinal repair. After CP administration in mice, the liver and immune system were severely damaged, and the structure of the gut microbiota also showed variation and imbalance. The relative abundance of Lactobacillus decreased significantly, while the abundance of Lactobacillus, Clostridiaceae, Lachnospiraceae, and Prevotelaceae increased significantly. This article tested the gut microbiota of mice and found that the CP group of AG fed mice had significant differences in gut microbiota. The gut microbiota composition of the CP+POP group is very similar to that of the CK group. The results indicate that the abundance of lactobacilli dominates. This similar result is consistent with the report in reference [11]. AG alone had a more obvious effect. In conclusion, the experimental data show that arabinogalactan can indeed improve the body's immunity.

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