Immunoprotection Effects of Radix Pseudostellariae Fibrous Root Extraction (RPFRE) on Mice and Its Relationship with Antioxidant Function

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Abstract **[Objective]** The paper was to explore the immunoprotection effects of Radix Pseudostellariae fibrous root extraction (RPFRE) on experimental mice and its relationship with antioxidant function. **[Method]** KM male mice were randomly divided into 4 groups based on RPFRE gradient concentration, and the immune function indexes and antioxidant indexes of mice were determined at 14 d post intragastric administration of RPFRE. The remaining mice in the experimental group were intraperitoneally injected with cyclophosphamide (CY) from the 15th to 17th day, and samples were collected to determine the above indexes on the 18th day. **[Result]** Intragastric administration of RPFRE for 14 d improved the spleen index, thymus index, T/B cell stimulation index and total antioxidant capacity (T-AOC) of normal mice, and extremely increased the thymus index and T-AOC ($P<0.01$). The T-AOC had extremely positive correlation with the spleen index, thymus index and B cell stimulation index ($P<0.01$), and had significantly positive correlation with the T cell stimulation index ($P<0.05$). From the 15th to 17th day of the experiment, the immune function indexes and antioxidant indexes of mice decreased and the malondialdehyde (MDA) content increased. The experimental groups of 0.1, 0.2 and 0.4 g/kg RPFRE promoted the immunoprotection and antioxidant effect of mice. RPFRE concentration had extremely positive correlation with 4 indexes of immune function and antioxidant indexes T-AOC and SOD ($P<0.01$), but had extremely negative correlation with MDA ($P<0.01$). There was a significantly positive correlation between immune function indexes and antioxidant indexes of mice after CY intervention ($P<0.05$ or $P<0.01$). **[Conclusion]** RPFRE can improve the immune function indexes and antioxidant indexes of normal mice and immunosuppressed mice and has immunoprotection effect on immunosuppressed mice, and there is a significant correlation between immune function indexes and antioxidant indexes of mice.

Keywords Radix Pseudostellariae fibrous root (RPFR); Extract; Mice; Immune; Antioxidant; Correlation

Radix Pseudostellariae fibrous root (RPFR) is the lateral root and tail root of Radix Pseudostellariae, accounting for about 10%–15% of the dry root weight of Radix Pseudostellariae. It is easy to obtain RPFR raw material for traditional Chinese medicine after dried processing. Radix Pseudostellariae is the dry tuber of *Pseudostellaria heterophylla* (Miq.) Pax, belonging to Caryophyllaceae, which was first recorded in *Bencao Congxin* (New Compilation of Materia Medica) in the Qing Dynasty, and is mainly produced in Fujian Province[1]. Zherong county is also known as "The hometown of Radix Pseudostellariae in China", with a long history of cultivation and utilization of Radix Pseudostellariae. Modern studies have found that Radix Pseudostellariae has both medicinal and nutritional effects, which is rich in polysaccharides, saponins and other medicinal components[2], and has the functions of invigorating the spleen and benefiting the lung[3], anti-oxidation[4], anti-inflammatory[5], enhancing immunity[6] and intestinal flora probiotics[7].

Immunity enhancement and antioxidant stress are the key factors to evaluate the health status of the body. A variety of Chinese herbal medicines and their extracts have been applied to the study of immune function and antioxidant effect in clinical practice. Zhao *et al.*[8] found that both *Astragalus membranaceus* and *Lycium chinense* can significantly improve the immune function and antioxidant effect of experimental animals. Kong *et al.*[9] reported that the extract of Radix Pseudostellariae had antioxidant effects *in vitro*. Feng *et al.*[10] put forward that Radix Pseudostellariae polysacchride injection had protective effect on immunosuppressed mice induced by cyclophosphamide. The authors had reported that the extracts of Radix Pseudostellariae had immune-enhancing and antioxidant effects[11–12]. Meantime, Zeng *et al.*[13] found that RPFR saponin can improve the immune function and OVA immunogenicity of mice and enhance the immune response ability of mice to OVA, play-

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ing an immune adjuvant role. Du *et al.*[14] reported that RPFRE could increase the levels of immunoglobulin and complement in mice to varying degrees, and simultaneously improve the serum antioxidant indexes of mice. However, studies on the immune function of normal and immunosuppressed mice and the relationship between immune function and antioxidant effect of RPFRE have not been reported. In this study, KM male mice were selected to study the immunoprotection effect and its relationship with antioxidant function of RPFRE at different gradient concentrations (0, 0.1, 0.2, 0.4 g/kg), which would provide more valuable scientific reference for the extensive application of Radix Pseudostellariae in animal health care.

1 Materials and Methods

1.1 Materials

1.1.1 RPFRE. RPFRE was provided by the research and development center of Fujian Beidi Pharmaceutical Co., Ltd. The raw materials of RPFR were collected from the main producing areas of Radix Pseudostellariae in Zherong County, Fujian Province. Polysaccharides and saponins are the main components of RPFRE.

1.1.2 Animals. Clean-grade male KM mice, 3–4 weeks old and weighing (20±2) g, were purchased from the Experimental Animal Center of Fujian Medical University.

1.1.3 Main reagents and instruments. ConA, fetal bovine serum, RPMI1640 culture solution, lipopolysaccharide (LPS), Hank's balanced salt solution (HBSS), methyl thiazolyl tetrazolium (MTT), dimethyl sulfoxide (DMSO), cyclophosphamide (CY), total antioxidant capacity detection kit, superoxide dismutase test kit, malondialdehyde test kit.

CO_2 incubator, X-22R desktop high-speed refrigerated centrifuge, SE202F electronic balance, ultra-clean table, Infinite M200 Pro multifunctional microplate reader, *etc*.

1.2 Methods

1.2.1 Grouping and treatment of animals. A total of 160 clean-grade male KM mice were adaptively fed for 5 d, which were randomly divided into 4 groups based on RPFRE gradient concentration (0, 0.1, 0.2 0.4 g/kg), with 40 mice in each group. The trial lasted 17 d, and the details are shown in Tab.1. Male KM mice were administered intragastrically with different concentrations of RPFRE from the 1st to 14th day. On the 15th day, samples were collected to determine the immune function indexes (spleen index, thymus index, T/B cell stimulation index) and antioxidant indexes (T-AOC, SOD, MDA). The remaining mice in the experimental group were intraperitoneally injected with cyclophosphamide (CY) from the 15th to 17th day, and samples were collected to determine the above indexes on the 18th day. Mice were allowed *ad libitum* to feed and drinking water during the test, and their weights were recorded. All mice were under the same management conditions.

1.2.2 Determination of immune organ index. On the 15th and 18th day of the test, 10 mice were randomly selected from each group for each experiment and weighed, and the mice were executed by cervical dislocation method. The spleen and thymus of mice were weighed respectively. The specific calculation methods of spleen index and thymus index were as follows.

Organ index=Organ weight (mg)/Body weight (g)

1.2.3 Determination of T/B cell stimulation index

1.2.3.1 Preparation of splenic lymphocyte suspension. Five mice from each experimental group were executed by cervical dislocation method, and the spleen was aseptically collected and placed in a sterilized mortar. After grinding, the sample was added with 3.75 mL of Hank's solution, mixed, and filtered to develop single spleen cell suspension. The suspension was rinsed with Hank's solution for 3 times, centrifuged at 1 500 r/min for 5 min, and added with 2 mL of RPMI1640 (complete culture medium) containing 10% fetal bovine serum. After vortex mixing and counting, the solution was adjusted to 7.5×10^6 cells/mL cell dilution for splenic cell proliferation.

1.2.3.2 Determination of splenic lymphocyte proliferation. Each well of a 96-well cell culture plate was added with 100 μL of the above spleen cell suspension, and the blank wells were added with 100 μL of RPMI1640 complete culture medium. The test wells were added with 100 μL of ConA (final concentration 5 μg/mL) and 100 μL of LPS (final concentration 10 μg/mL). The spleen lymphocyte of each mouse had four replicate wells. After cultured in 5% CO_2 incubator for 44 h, each well was added with 50 μL of MTT solution, followed by 4 h culture and centrifugation at 1 000 r/min for 5 min. After the supernatant was discarded, each well was added with 150 μL of DMSO, and the plate was oscillated at low speed for 5 min. After zero setting with reagent blank well, the absorbance of each well was determined at the wavelength of 570 nm, and the T/B lymphocyte stimulation index was

Tab.1 Experimental design

Drug	Dose// g/kg	Time of intragastric administration	Time of CY intraperitoneal injection
RPFRE	0.0	The 1st to 14th day	The 15th to 17th day
RPFRE	0.1	The 1st to 14th day	The 15th to 17th day
RPFRE	0.2	The 1st to 14th day	The 15th to 17th day
RPFRE	0.4	The 1st to 14th day	The 15th to 17th day

calculated.

T/B lymphocyte stimulation index = OD value of test well/OD value of blank well.

1.2.4 Collection of blood samples and determination of antioxidant indexes. The blood samples of mice were collected by enucleation and centrifuged at 3 000 r/min for 10 min. The serum was collected and stored at −20 ℃. The T-AOC, SOD and MDA in the serum were detected according to the instructions and requirements of the kit, and the values were calculated.

1.3 Statistical analysis All data were analyzed by ANOVA via SPSS 25 software, and multiple comparison analysis and index correlation analysis were performed by LSD method. The results were expressed as mean ± standard deviation. $P<0.05$ indicated significant difference and $P<0.01$ indicated extremely significant difference.

2 Results and Analysis

2.1 Effects of RPFRE on immune function indexes of normal mice As shown in Fig.1 (A-1, B-1), RPFRE improved the spleen index and thymus index levels of normal mice at 14 d post intragastric administration. Compared with 0 g/kg RPFRE, 0.1–0.4 g/kg RPFRE significantly increased the thymus index of mice ($P<0.05$); 0.1 g/kg RPFRE significantly increased the spleen index of mice ($P<0.05$); there was no significant increase in spleen index with the increase of RPFRE concentration ($P>0.05$), while the concentration of RPFRE significantly affected the thymus index of normal mice. As shown in Fig.1 (C-1, D-1), RPFRE improved the B cell stimulation index and T cell stimulation index of normal mice at 14 d post intragastric administration. 0.1 g/kg RPFRE increased the B cell stimulation index of normal mice; 0.1 and 0.2 g/kg RPFRE significantly increased the T cell stimulation index of normal mice ($P<0.05$); and RPFRE significantly affected the T cell stimulation index of normal mice.

2.2 Effects of RPFRE on immune function of immunosuppressed mice As shown in Fig.1 (A-2, B-2), the thymus index and spleen index decreased after intragastric administration of RPFRE in normal mice for 14 d and CY immunosuppression. 0.1 –0.4 g/kg RPFRE extremely improved the thymus index and spleen index of immunosuppressed mice ($P<0.01$). The thymus index and spleen index were dose-dependent on the concentration of RPFRE, and the concentration of RPFRE had significant effect on the immune organ index of immunosuppressed mice. According to Fig.1 (C-2, D-2), compared with 0 g/kg RPFRE, 0.1–0.4 g/kg RPFRE improved the B cell stimulation index and T cell stimulation index of im-

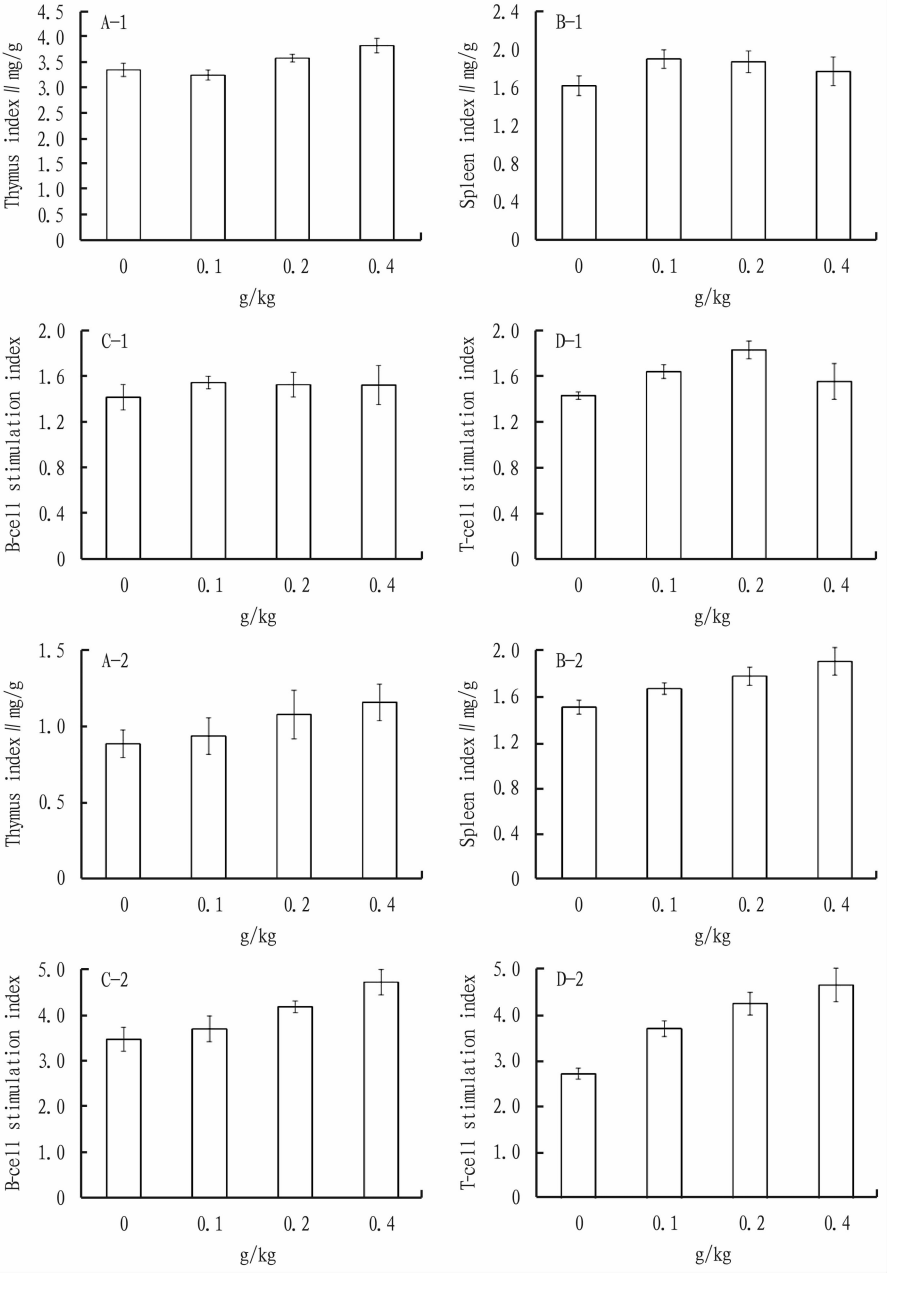

Fig.1 Effect of RPFRE on immune function indexes of mice

munosuppressed mice, and had a certain dose dependence on the concentration of RPFRE. The concentration of RPFRE significantly affected the proliferation of spleen lymphocytes in immunosuppressed mice. RPFRE showed a certain immunosuppressive protection effect on immunosuppressed mice.

2.3 Effects of RPFRE on antioxidant indexes of normal mice As shown in Fig.2 (A-1, B-1), RPFRE improved the serum T-AOC and SOD activity of normal

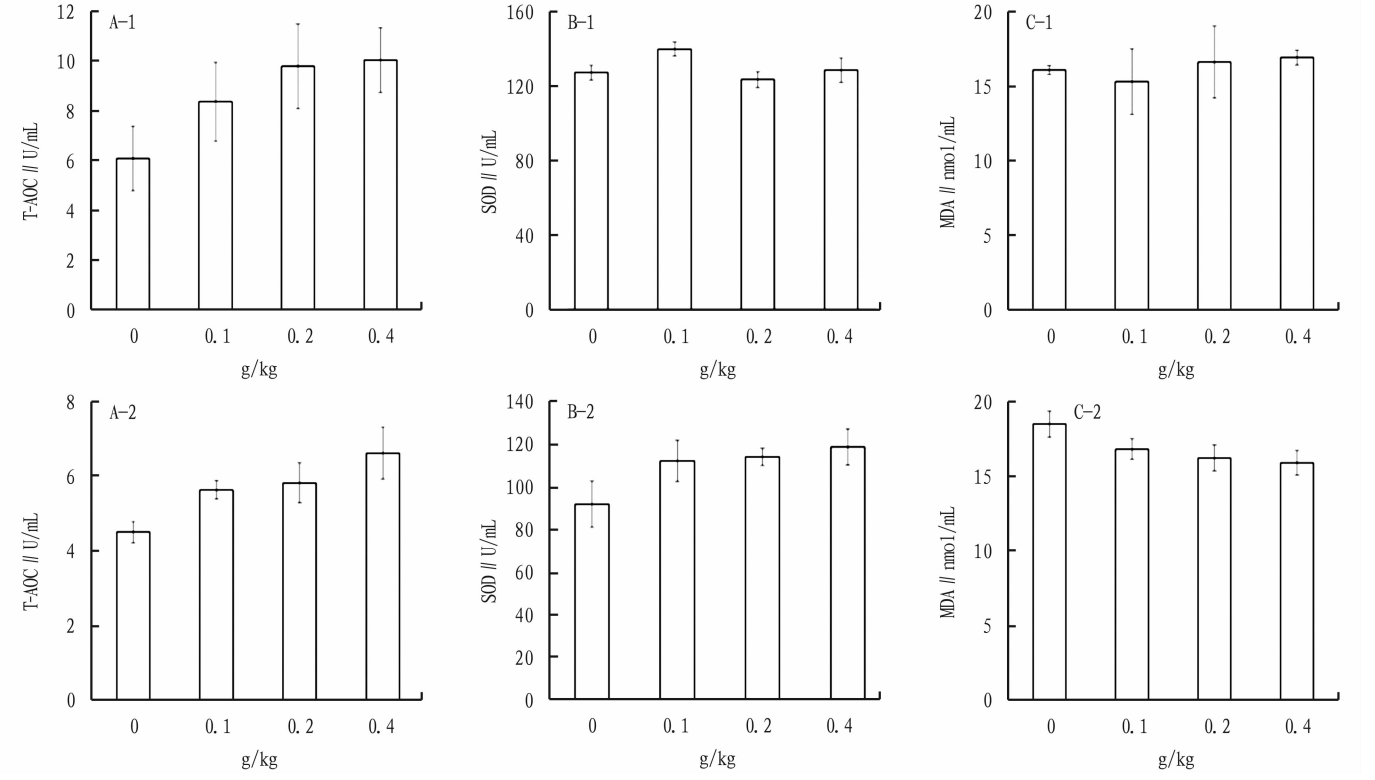

Fig.2 Effects of RPFRE on antioxidant indexes of normal mice

mice at 14 d post intragastric administration. Compared with 0 g/kg RPFRE, 0.1–0.4 g/kg RPFRE extremely increased the T-AOC activity of normal mice ($P<0.01$), while 0.1 g/kg RPFRE only significantly increased the SOD activity of normal mice ($P<0.05$). The increase of RPFRE concentration had no significant effect on SOD activity, but slightly decreased the SOD activity ($P>0.05$). As shown in Fig.2 (C-1), only 0.1 g/kg RPFRE reduced the MDA content of normal mice after intragastric administration, while 0.2 and 0.4 g/kg RPFRE increased the MDA content, but there was no significant difference between three experimental data and the data of 0 g/kg RPFRE ($P>0.05$). Intragastric administration of RPFRE increased the T-AOC and SOD activity in the serum of normal mice, but had no significant impact on MDA content.

2.4 Effects of RPFRE on antioxidant indexes of immunosuppressed mice As shown in Fig.2 (A-2 and B-2), the serum T-AOC and SOD activity of normal mice decreased significantly after intragastric administration of RPFRE in normal mice for 14 d and CY immunosuppression ($P<0.05$). 0.1–0.4 g/kg RPFRE extremely increased the T-AOC and SOD activity of immunosuppressed mice ($P<0.01$). T-AOC had a certain dose dependence on RPFRE concentration. There was no significant difference in SOD activity between 0.2 and 0.4 g/kg RPFRE ($P>0.05$), and the concentration of RPFRE significantly affected T-AOC and SOD activity of immunosuppressed mice. As shown in Fig.2 (C-2), compared with 0 g/kg RPFRE, 0.1–0.4 g/kg RPFRE extremely reduced the MDA content of immunosuppressed mice ($P<0.01$), and it had a certain dose dependence on RPFRE concentration. RPFRE concentration significantly decreased the MDA content of immunosuppressed mice. RPFRE enhanced the antioxidant activity of immunosuppressed mice.

2.5 Correlation analysis of immune function and antioxidant effect of mice induced by RPFRE The correlation analysis results of immune function indexes and antioxidant indexes of normal mice at 14 d post intragastric administration are shown in Tab.2. The results showed that the thymus index and T-AOC of normal mice were extremely positively correlated with RPFRE concentration ($P<0.01$), and the correlation coefficients were 0.821 and 0.649, respectively. There was an extremely positive correlation between

Tab.2 Correlation analysis of immune indexes and antioxidant indexes of normal mice

Index	Spleen index	Thymus index	T cell stimulation index	B cell stimulation index	T-AOC	SOD	MDA
Spleen index	1						
Thymus index	0.181	1					
T cell stimulation index	0.434**	0.098	1				
B cell stimulation index	0.259	−0.049	0.028	1			
T-AOC	0.509**	0.466**	0.333*	0.414**	1		
SOD	0.226	−0.407	−0.198	0.178	0.054	1	
MDA	−0.115	−0.027	0.084	−0.106	−0.140	−0.263	1
EFRPH concentration	0.214	0.821**	0.223	0.248	0.649**	−0.158	0.255

Note: * and ** represent significant difference and extremely significant difference at 0.05 and 0.01 levels, respectively; the same below.

Tab.3 Correlation analysis of immune indexes and antioxidant indices of immunosuppressed mice

Index	Spleen index	Thymus index	T cell stimulation index	B cell stimulation index	T-AOC	SOD	MDA
Spleen index	1						
Thymus index	0.579**	1					
T cell stimulation index	0.829**	0.570**	1				
B cell stimulation index	0.748**	0.614**	0.798**	1			
T-AOC	0.700**	0.661**	0.788**	0.710**	1		
SOD	0.676**	0.453**	0.677**	0.541**	0.621**	1	
MDA	−0.641**	−0.370*	0.751**	−0.626**	−0.734**	−0.527**	1
EFRPH concentration	0.860**	0.659**	0.892**	0.903**	0.812**	0.649**	−0.685**

spleen index and T cell stimulation index of normal mice ($P<0.01$), and the correlation coefficient was 0.434. T-AOC of normal mice had a positive correlation with spleen index, thymus index, T-cell stimulation index and B-cell stimulation index, and the correlation coefficients were 0.509, 0.466, 0.333 and 0.414, respectively, ($P<0.01$). The results showed that there was a positive correlation between total antioxidant capacity and immune function of normal mice after intragastric administration of RPFRE.

The correlation analysis results of immune function indexes and antioxidant indexes of mice after intragastric administration of RPFRE for 14 d in normal mice and CY immunosuppression are shown in Tab.3. The results showed that the concentration of RPFRE had significant correlation with the indexes of immunosuppressed mice ($P<0.01$), and had negative correlation with MDA content. The spleen index and thymus index of immunosuppressed mice were positively correlated with T cell stimulation index and B cell stimulation index ($P<0.01$). The antioxidant indexes T-AOC and SOD of immunosuppressed mice had extremely negative correlation with MDA content ($P<0.01$). There was an extremely significant correlation between immune function indexes and antioxidant indexes of immunosuppressed mice ($P<0.01$), and there was a negative correlation between immune function indexes (except T cell stimulation index) and MDA content. The results showed that there was a significant correlation between the immune function indexes and antioxidant indexes of mice after intragastric administration of RPFRE in normal mice for 14 d and CY immunosuppression.

3 Discussion

Thymus and spleen are important immune organs. Thymus index and spleen index can directly reflect its immune function, and proliferation of spleen cells also reflects the body's humoral immunity and cellular immunity level to a certain extent [15]. Intragastric administration of RPFRE for 14 d significantly increased the thymus index of normal mice ($P<0.05$), and there was an extremely positive correlation between spleen index and T cell stimulation index ($P<0.01$). After intragastric administration of RPFRE in normal mice for 14 d and CY immunosuppression, the thymus index, spleen index and splenic lymphocyte proliferation decreased in immunosuppressed mice. With the increase of the concentration of RPFRE, the

index values were increased and improved, showing a certain immunosuppressive protection effect on immunosuppressed mice. The results indicated that RPFRE increased the immune capacity of normal mice and immunosuppressed mice, and there was a certain dose and concentration dependence on RPFRE for immunosuppressed mice.

In antioxidant studies, oxygen free radicals can be produced by enzyme system and non-enzyme system in body metabolism, which easily leads to lipid peroxidation reaction of cell membrane and produces MDA and other substances, triggering the body's cell metabolism disorders and biological barrier, and causing or inducing the symptoms of inflammation or damage of body tissues. Therefore, the antioxidant level of the body's defense system plays a vital role. Studies have proved that serum T-AOC, SOD and MDA can reflect the balance between oxidation and antioxidant in the body[16], and MDA, as a landmark product of lipid peroxidation, can directly reflect the level of oxidative stress in the body. The results showed that RPFRE significantly improved the T-AOC of normal mice after intragastric administration for 14 d (P<0.05), and T-AOC had significantly positive correlation with immune function indexes including spleen index, thymus index, T cell stimulation index and B cell stimulation index. The serum T-AOC and SOD activity of immunosuppressed mice significantly increased after intragastric administration of RPFRE in normal mice for 14 d and CY immunosuppression, and the MDA content decreased remarkably. The spleen index and thymus index of immunosuppressed mice were positively correlated with T cell stimulation index and B cell stimulation index, respectively. The antioxidant indexes T-AOC and SOD were negatively correlated with MDA, and the immune function indexes of immunosuppressed mice had significant correlation with antioxidant indexes (P<0.01). The results showed that RPFRE increased the activity of antioxidant enzymes in normal mice and immunosuppressed mice, reduced the level of lipid peroxidation, and protected cells from oxidative damage. In addition, after CY immunosuppression in normal mice, the activity of serum antioxidant enzyme decreased significantly, and MDA content increased significantly, indicating that CY led to the decrease of antioxidant capacity and the increase of peroxidation in immunosuppressed mice. Combined with the reduction of immune function indexes of immunosuppressive mice, it showed that CY had a bilateral inhibition of immune function and antioxidant effect of immunosuppressive mice. RPFRE improved the immune function index and antioxidant index of normal mice and immunosuppressed mice, which had positive effect on the total antioxidant capacity and immune function of normal mice, and had an immunoprotection effect on immunosuppressed mice. The immune function indexes were significantly correlated with antioxidant indexes, indicating that RPFRE was closely related to antioxidant function, redox equilibrium and immune function of mice, and antioxidant function mutually interacted with immune function. The results will provide some basic data support for further study on the mechanism of RPFRE in interaction between immunity and antioxidant in the body in the future, and also provide a new idea for RPFRE to enhance immunity by enhancing antioxidant level of the body.

4 Conclusions

RPFRE significantly increased the thymus index and T-AOC of normal mice after intragastric administration for 14 d (P<0.05). The spleen index was positively correlated with T cell stimulation index (P<0.01). T-AOC was positively correlated with spleen index, thymus index, T cell stimulation index and B cell stimulation index, respectively. Intragastric administration of RPFRE in normal mice for 14 d and CY immunosuppression significantly increased the thymus index, spleen index, B cell stimulation index and T cell stimulation index of immunosuppressed mice (P<0.01). In addition, it was dose-dependent with RPFRE, and the T-AOC and SOD activity of immunosuppressed mice showed similar trend. RPFRE had a significant effect on the decrease of MDA content. Spleen index and thymus index were positively correlated with T cell stimulation index and B cell stimulation index, respectively. T-AOC and SOD were negatively correlated with MDA, and immune function indexes had extremely significant correlation with antioxidant indexes (P<0.01). In conclusion, RPFRE can improve the immune function index and antioxidant index of normal mice and immunosuppressed mice, which has a positive effect on the total antioxidant capacity and immune function of normal mice, and has immune protection effect on immunosuppressed mice, and immune function indexes are significantly correlated with antioxidant indexes.

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