

Downregulation of CD38, CD95, and PD-1 Expression in T Cells of Aging Mice by Intervention of *Polygala fallax* HemsI

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Abstract. The immune system plays an essential role in aging. *Polygala fallax* HemsI. (PFH) enhances the immune response and promotes immune function. The study aimed to investigate whether PFH had a positive effect on the immune function of aging mice to delay aging. Kunming mice were selected to establish an aging mouse model by intraperitoneal injection of D-galactose at 500 mg/kg/day for 6 weeks. Astragalus membranaceus, which has been used as one of the positive control groups. After modeling, A. membranaceus low, medium, and high doses of PFH were administered by gavage for 4 weeks, Serum superoxide dismutase (SOD) and malondialdehyde (MDA) levels were evaluated using enzyme-linked immunosorbent assay. Flow cytometry was used to determine T-cell subsets and the expression of CD38, CD95, and PD-1 in peripheral blood. Prism 9.5.1 statistical software for classification analysis was used with all data expressed in ($\bar{x} \pm s$). One way ANOVA was further used to detect statistically significant differences between group. Results revealed that serum SOD activity was decreased and MDA content was elevated in the MODEL group compared with those in the CONTROL group. A. membranaceus and PFH increased SOD activity and decreased MDA content. Furthermore, the expression of CD38, CD95, and PD-1 in CD4+ and CD8+ T cells was increased in the MODEL group compared with those in the CONTROL group. CD38, CD95, and PD-1 expression decreasing trend after the administration of A. membranaceus and PFH. Among them, CD38, CD95, and PD-1 were the most significantly reduced in PFH-L group. It is therefore concluded that PFH had a positive effect on CD4+ and CD8+ T-cell function in aging mice, which could delay aging to a certain extent.

1. Introduction

Recently, the problem of population aging has become increasingly prominent, and with the national conditions of China, aging will remarkably affect the population of China at a large scale and rapid speed [1]. Aging is an inevitable biological process, which is the decline of the body at the molecular and cellular levels, particularly organ function, and is a comprehensive and complex physiological and pathological process [2]. Aging is mainly characterized by a progressive decline in biological integrity and its compensatory mechanisms and immune dysregulation [3]. The current research focus lies in finding ways to reduce the repetition and delay the aging process, thereby enhancing the body's disease resistance and improving the quality of life for the elderly. Therefore, exploring the basic biology and mechanisms of aging is essential for developing interventions to prevent and treat its complex phenotype.

Immunosenescence, which refers to the aging of the immune system, is a complex and multifaceted phenomenon that affects both natural and acquired immunity as a result of the aging process [4]. It involves various factors such as changes in aging-related characteristics, decline in immune organ functions, and

alterations in immune cell subpopulations. These alterations play a substantial role in the emergence and advancement of age-related illnesses. Despite the progress made in understanding immunosenescence, its precise mechanisms are still not completely understood. In other words, there is still much to learn about the specific mechanisms underlying immunosenescence, despite the advancements in our understanding of the topic.

T cell are a crucial cell type in the immune system, and their activation and depletion states are critical for the maintenance and recuperation of immune function [5]. With aging, T cell function declines, leading to increased susceptibility to infections and cancer. CD38, CD95, and PD-1 are markers that have been associated with T cell senescence and dysfunction. CD38 is a transmembrane glycoprotein that is expressed on the surface of T cells and has been shown to be involved in T cell activation and proliferation [6]. CD95, also known as Fas, is a cell surface receptor that induces apoptosis in T cell [7]. PD-1 is a cell surface receptor that negatively regulates T cell activation and has been shown to be involved in T cell exhaustion [9].

Traditional Chinese medicine (TCM) is characterized by nourishing life, and its anti-aging effects are receiving increasing attention. TCM believes that aging is caused by the deficiency of one's five organs, deficiency of qi and blood, and imbalance of yin and yang. *Polygala fallax*

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Hemsl. (PFH) is a common folk medicinal herb used by ethnic minorities in Guangxi and is also widely used in China for treating chronic diseases. Studies have shown that PFH significantly reduces blood lipids, particularly plasma triglycerides [8]. Moreover, PFH has anti-hepatocellular carcinoma activity. Furthermore, PFH combined with compound Sanqi granules has been reported to reduce glomerulonephritis by regulating glomerular thylakoid cell proliferation and apoptosis [10]. However, the effect of PFH on immune cells in aging is not yet known.

The objective of this study was to investigate the potential beneficial effects of PFH on immune function in an aging mice model. To achieve this, the mice were randomly divided into 6 groups. Molecular markers of peripheral blood T-cell activation and apoptosis expression were measured to assess the impact of PFH on delaying aging.

2. Materials and methods

2.1. Animals and treatments

Twenty-four Kunming mice were purchased from the Experimental Animal Center of Guangxi Medical University. The experimental animal license number was SCXK Gui 2009-0002, and the experimental animal use license was SYKG Gui 2009-2005. The mice were acclimatized for 1 week before starting the experiment. The mice were then randomly assigned to different groups: the healthy control (CONTROL) group, aging model (MODEL) group, Astragalus membranaceus (A) group, high-dose PFH (PFH-H) group, medium-dose PFH (PFH-M) group, and low-dose PFH (PFH-L) group. Four animals were used in each group. After that, 500-mg/kg/d D-galactose was injected intraperitoneally continuously for 42 days to establish the aging model. The treatment of the mice in the CONTROL group was as follows: D-galactose used in the operation of the MODEL group was replaced with the same dose of saline, and all other operation procedures and breeding conditions were the same as those in the MODEL group, and the same behavioral changes in the mice were observed after treatment.

After modeling, Astragalus membranaceus and low, medium, and high doses of PFH were administered to the mice. A group was given an aqueous decoction of Astragalus membranaceus containing 15 g/kg of raw herbs. The PFH-H group was given an aqueous decoction of PFH containing 15 g/kg of raw herbs. The PFH-M group was given an aqueous decoction of PFH containing 7.5 g/kg of raw herbs. The PFH-L group was given an aqueous decoction of PFH containing 3.75 g/kg of raw herbs. The CONTROL and MODEL groups were given the same volume of saline by gavage. At 4 weeks of administration, 1 mL of peripheral blood was collected after 12 h of fasting without water to prepare for subsequent experiments.

2.2. Enzyme-linked immunosorbent assay (ELISA)

Mouse serum was collected to assess the levels of superoxide dismutase (SOD) and malondialdehyde (MDA). The SOD and MDA levels in the serum were measured using quantitative ELISA kits (SOD kit: ml643059-1, MLBIO; MDA kit: mlsh0387, MLBIO) following the instructions provided by the manufacturer.

2.3. Flow cytometry (FCM)

After obtaining the mouse peripheral blood mononuclear cells, 50 μ L of each pre-configured cell suspension was taken. Then, PE Hamster Anti-Mouse CD3e, PerCP/Cyanine5.5 Rat Anti-Mouse CD4, APC Rat Anti-Mouse CD8, FITC Rat Anti-Mouse CD38, BV510 Hamster Anti-Mouse CD95, and BV421 Hamster Anti-Mouse CD279 (PD-1) were added to each tube at a concentration of 1 μ L. The mixture was blown and mixed well and then incubated at 4 $^{\circ}$ C in the dark for 30 minutes. Next, 1 mL PBS was added to each tube, blown and mixed well, and then centrifuged at room temperature at 3200 rpm for 5 minutes. The supernatant was discarded, and 500 μ L PBS was added to each tube, blown and mixed well, and then centrifuged at room temperature at 3200 rpm for 5 minutes. This step was repeated once more. The supernatant was discarded, and 500 μ L PBS containing 1% paraformaldehyde dilution solution was added to each tube to resuspend the cells. Finally, the correlation between data from different groups was detected and analyzed.

2.4. Statistical analysis

Graphpad Prism 9.5.1 statistical software was utilized for classification analysis. The data expressed in ($\bar{x} \pm s$) were compared using the One-way ANOVA method. Results indicated that any $P < 0.05$ implied statistically significant differences between the groups.

3. Results and discussion

3.1. Established D-galactose aging model of mice by detecting the expression of oxidative indicators SOD and MDA.

The success of the D-galactose-induced aging model in mice has been confirmed. Results showed that the MODEL group had decreased serum SOD activity and increased MDA content compared to the CONTROL group. After treatment with *A. membranaceus* and *Polygala fallax* Hemsl., the treated group showed increased SOD activity and decreased MDA content (Table 1, Figure 1).

Table 1: Superoxide Dismutase (SOD) Activity and Increase in Malondialdehyde (MDA) Expression in Serum

Groups	SOD(U/mL)	MDA(nmol/mL)
CONTROL	9.53±1.07	323.50±52.87
MODEL	5.55±0.71	810.50±98.31
A	7.09±0.86	471.17±52.82
PFH-H	6.98±0.74	651.17±73.84
PFH-M	7.21±0.87	507.17±64.87
PFH-L	8.42±0.84	378.83±41.89

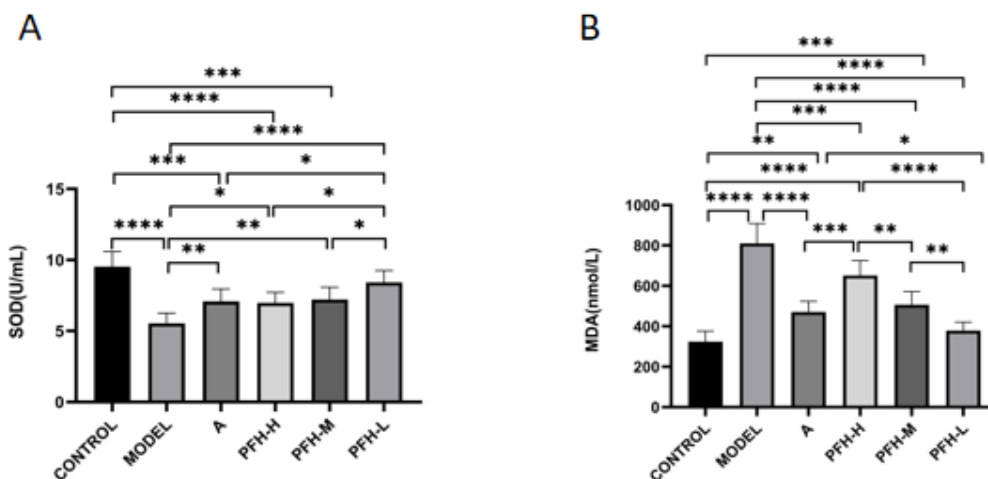


Figure 1: A: ELISA determination of serum superoxide dismutase (SOD) expression levels in mice. B: ELISA determination of serum malondialdehyde (MDA) expression level in mice. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

3.2. Detection the expression of activation marker CD38 and exhaustion marker CD95 in CD4+T cells.

The expression of activated and depleted molecules CD38 and CD95 on mouse peripheral blood CD4+ cells were

detected. The MODEL group showed higher levels of CD38 and CD95 expression on CD4+T cells compared to the CONTROL group. However, after the administration of *A. membranaceus* and *Polygala fallax* Hemsl., the expression of CD38 and CD95 on CD4+T cells decreased, indicating a reduction in the repetition rate. Notably, the PFH-L group demonstrated a significant reduction in the expression of CD38 and CD95 (Figure 2).

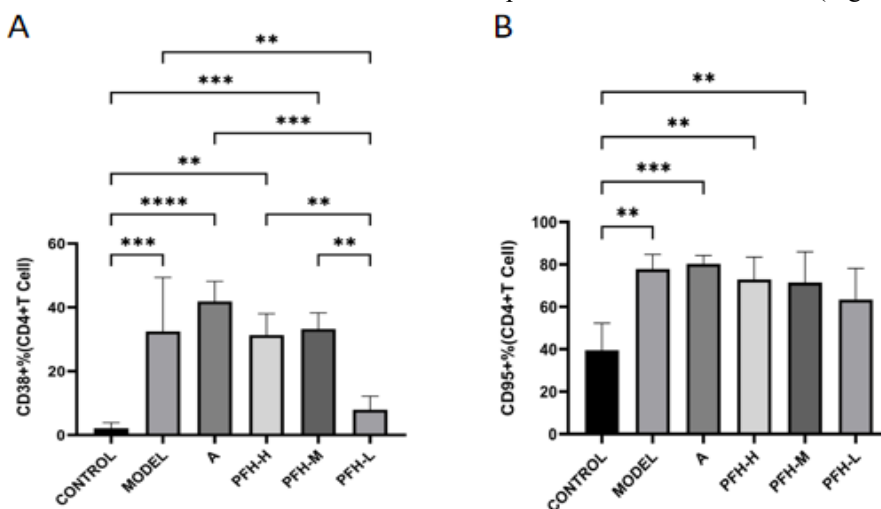


Figure 2: A: Flow cytometry detection of CD4+CD38+ T cells expression in peripheral blood. B: Flow cytometry detection of CD4+CD95+ T cells expression in peripheral blood. * P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

3.3. Detection the expression of activation marker CD38 and exhaustion marker CD95 in CD8+T cells.

The levels of CD38 and CD95 expression in CD8+ T cells of mouse peripheral blood were found to be elevated in

the MODEL group compared to the CONTROL group. After the administration of *A. membranaceus* and *Polygala fallax* Hemsl., the expression of CD38 and CD95 in mouse CD8+ T cells exhibited a decrease. The PFH-L group showed the most pronounced reduction in CD38 and CD95 expression, indicating a significant decrease in their levels. (Figure 3).

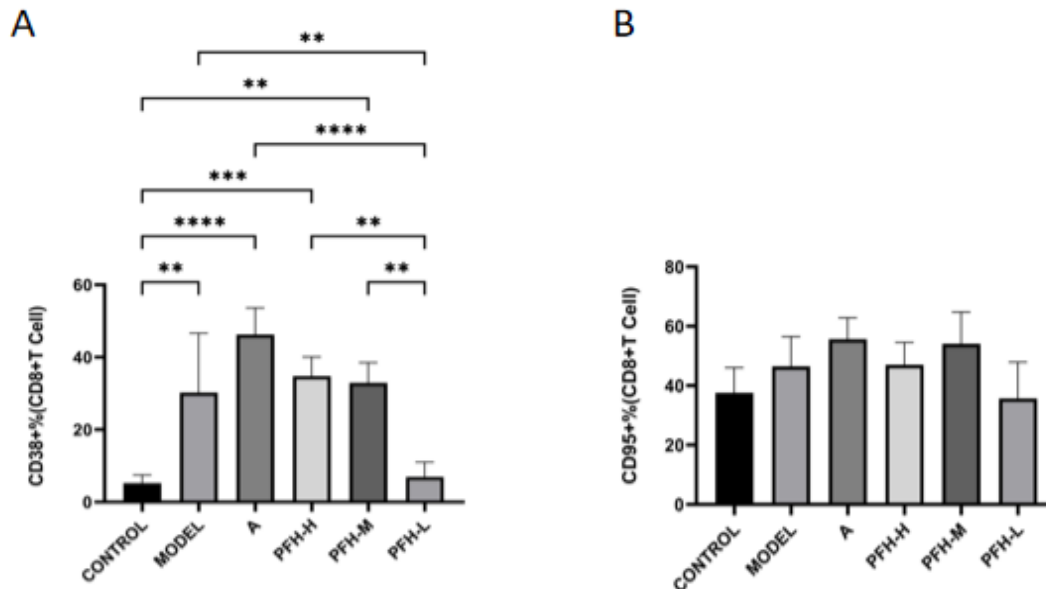


Figure 3. A: Flow cytometry detection of CD8+CD38+T cells expression in peripheral blood. B: Flow cytometry detection of CD8+CD95+T cells expression in peripheral blood.
 *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

3.4. Detection the expression of programmed death factor PD-1 in CD4+T and CD8+T cells in D-galactose aging model mice.

The expression of programmed death-1 (PD-1) on CD4+ and CD8+ T cells in the peripheral blood of mice was investigated. The expression of PD-1 in CD4+ and CD8+ T cells was found to be higher in the mice of the MODEL

group compared to those in the CONTROL group. Following treatment with *A. membranaceus* and *Polygala fallax* Hemsl., groups A, PFH-H, and PFH-M showed a slight elevation in PD-1 expression in both CD4+ and CD8+ T cells. However, the mice in the PFH-L group showed a significant decrease in PD-1 expression, which aligns with the previously mentioned trend of CD38 expression (Figure 4).

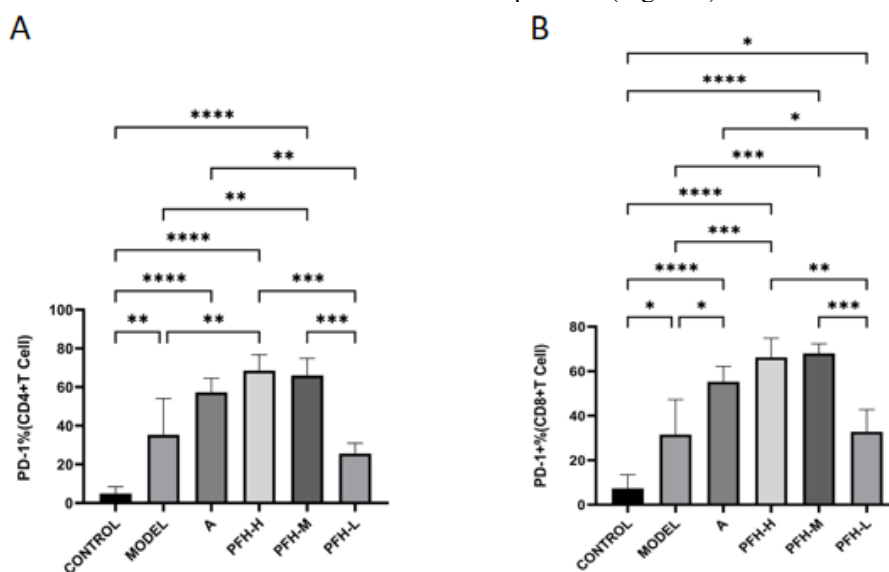


Figure 4. A: Flow cytometry detection of CD4+PD-1+T cells expression in peripheral blood. B: Flow cytometry detection of CD8+PD-1+T cells expression in peripheral blood.
 *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001.

4. Conclusion

In this study, CD38, CD95, and PD-1 expression in peripheral blood CD4⁺ and CD8⁺ T cells was elevated in aging mice. Increased CD38 expression indicates that these cells have been activated and are in a higher metabolic state. Increased CD95 expression indicates that these cells are under strong activation and stress and that these cells are at risk of apoptosis, indicating an imbalance in their metabolism that decreases the immune response. PD-1 expression was found to be elevated in CD4⁺ and CD8⁺ T cells from aging mice. This increase in PD-1 expression may be attributed to the age-related decline in immune system function, leading to reduced T cell activity and heightened PD-1 expression. However, the use of PFH-L decreased the expression of CD38, CD95, and PD-1. It is suggested that PFH could inhibit the overactivation and apoptosis pathways of T cells in the aging process of mice, which may help protect immune cells in aging mice from the influence of overactivation and apoptosis, enhance the immune response level of T cells, and delay aging to a certain extent. Therefore, further exploration of the mechanisms and effects of these changes will help understand the occurrence and development of immunosenescence and provide new ideas and methods for preventing and treating immune-related diseases in the elderly.

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