Journal of Experimental and Clinical Application of Chinese Medicine

ORIGINAL RESEARCH

Open Access

Ganoderma lucidum Polysaccharides Promote Thymic Regeneration in Starvation Mice

Xiaoyu Lai [1,#], Yun Xin [1,#], Jianli Gao [1,*]

[1.] School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, 311499 Hangzhou, Zhejiang, China

# The authors contributed equally to this work.

DOI: https://doi.org/10.62767/jecacm701.5528

Keywords

Ganoderma lucidum polysaccharides

Starvation-induced thymic involution

Thymic regeneration

Immunosenescence

Thymic epithelial cells

* **Correspondence**

Jianli Gao

School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, 311499 Hangzhou, Zhejiang, China

E-mail: jianligao@zcmu.edu.cn

Received: 31 December 2025

Revised: 9 February 2026

Accepted: 27 March 2026

Published: 31 March 2026

Journal of Experimental and Clinical Application of Chinese Medicine **2026; 7(1): 55-66.**

Abstract

Background: Thymic functional decline is closely associated with immunosenescence. *Ganoderma lucidum* polysaccharides (GLP) are major immunoactive constituents of Ganoderma (Lingzhi, a well-known anti-aging herbs), but its effect on thymic regeneration remains limited. **Method:** An acute starvation-induced thymic involution model (induced by 72 h fasting) was established, female BALB/c mice were then refeeding with GLP (10/30/100 mg/kg, oral gavage) or metformin (100 mg/kg, i.p.) for 14 days. General behavior, spontaneous activity, thymus/spleen indices and thymic corticomedullary architecture was examined. The distribution of thymic epithelial cell (TEC) was assessed by immunofluorescence markers CK5/CK8, and thymic T-cell subsets (DN, DP, $CD4^+SP$, $CD8^+SP$) were quantified by flow cytometry. **Results:** A 72 h fast induced the thymic index decreasing from 0.17 ± 0.01% to 0.09 ± 0.03% ($p < 0.05$), and increasing to 0.18 ± 0.03% after refeeding, but its corticomedullary architecture and the distribution of T-cell subsets were not fully restored. Compared with the Regeneration group, GLP treatment nice have a clearer corticomedullary demarcation. Immunofluorescence indicated that GLP (particularly GLP-L/GLP-H) and metformin enhanced CK5- and CK8-positive cells and increased the density of the reticular network. Flow cytometry showed increased proportions of DN T cells and $CD8^+SP$ cells in the treatment groups relative to the spontaneous-regeneration group. **Conclusion:** GLP repairs the structure and function of thymus in acute starvation-induced thymic involution, indicating potential value for counteracting thymus-associated immunosenescence and enhancing thymic regeneration.

1 Introduction

The thymus is the primary lymphoid organ in mammals, serving as a critical site for the development, differentiation, and establishment of immune tolerance in mature T cells. It plays a pivotal role in maintaining the homeostasis of the peripheral T-cell pool and its diversity. Within the thymus, a complex microenvironment constructed by thymic epithelial cells (TECs) drives the differentiation of bone marrow-derived precursor cells into functionally mature T cells, thereby contributing to adaptive immune responses, pathogen recognition, and tumor immunosurveillance [1]. However, the thymus undergoes progressive age-related involution after puberty, characterized by disruption of tissue architecture, alterations in cellular composition, and reduced output of new T cells. This involution represents a major mechanism of immunosenescence and is prominently associated with aging, chronic infections, immune deficiency, and impaired immune reconstitution following chemotherapy [2]. Therefore, elucidating the molecular mechanisms underlying thymic involution and identifying strategies to promote thymic regeneration are of significant theoretical and clinical importance for reversing immunosenescence and enhancing disease resistance in elderly and immunocompromised populations.

Ganoderma lucidum (Reishi or Lingzhi) is an important traditional Chinese medicinal mushroom with a history of use spanning over a thousand years. Modern pharmacological studies have demonstrated that *G. lucidum* is rich in bioactive components, including polysaccharides and triterpenoids, which exhibit broad immunomodulatory, anti-inflammatory, antitumor, and antioxidant effects, making them promising candidates for regulating immune function and ameliorating age-related pathological processes [3]. Recent clinical and interventional studies have shown that supplementation with dried *G. lucidum* extracts can improve T lymphocyte function in elderly individuals, enhance lymphocyte proliferation, and upregulate key immunoregulatory molecules, suggesting a potential role in alleviating immunosenescence [4]. These findings support the value of *G. lucidum* as a natural immunomodulator in ameliorating thymic involution and age-associated immune decline.

Polysaccharides have long been recognized as major bioactive constituents in natural products. *G. lucidum* polysaccharides (GLPs), derived from fungal sources, are heterogeneous polymers composed of more than ten monosaccharides linked through glycosidic bonds. To date, over 220 distinct polysaccharides have been isolated from *G. lucidum*, the majority of which consist of β-glucans, with only a small fraction comprising α-glucans; the latter generally exhibit little or no biological activity [5]. Pharmacological studies by Guo and colleagues demonstrated that GLPs significantly reduce disease activity indices, total tumor number, and tumor size in azoxymethane/dextran sulfate sodium (AOM/DSS)-induced models of colitis and colorectal cancer. Furthermore, GLPs ameliorated AOM/DSS-induced gut dysbiosis by inhibiting the Toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88)/nuclear factor-κB (NF-κB) signaling pathway [6]. Han et al. reported that GLPs strongly stimulate proliferation of murine splenic B cells, which are subsequently phagocytosed by macrophage-like RAW 264.7 cells, triggering biological responses such as nitric oxide production [7]. Liu et al. demonstrated that GSP-2, a GLP, functions as a TLR4 agonist, inducing TLR4 overexpression in a dose-dependent manner [8].

GLP exhibits pharmacological activities including antioxidant, anti-immunosenescence, and antitumor effects; however, its impact on the thymus has not been investigated. This study aims to further assess the effects of GLP on thymic regeneration by

comparing behavioral parameters, organ indices, and histopathological changes between control and GLP-treated mice.

2 Materials and methods

2.1 Extraction of *Ganoderma lucidum* polysaccharides (GLP)

The *Ganoderma lucidum* slices were purchased from the Chinese Medicine Pharmacy of the First Affiliated Hospital of Zhejiang University School of Medicine. The Ganoderma was crushed, and 100 g of Ganoderma powder was weighed and dissolved in distilled water at a liquid-to-material ratio of 1: 28.9. The mixture was soaked for 10.3 hours, followed by a 3.2-hour heating in a water bath at 89 ℃. Extraction was performed three times, and the filtrates were combined. The combined filtrates were centrifuged at 4000 rpm for 15 minutes, and the supernatant was collected. The supernatant was concentrated to one-quarter of its original volume using a rotary evaporator, and ethanol was added to precipitate the polysaccharides (ethanol concentration to 80%). The solution was then placed in a 4 ℃ refrigerator overnight. After overnight incubation, the solution was centrifuged at 4000 rpm for 15 minutes to collect the precipitate. Finally, the precipitate was freeze-dried for 24 hours using a lyophilizer, yielding 0.66 g of *Ganoderma lucidum* polysaccharides.

2.2 Animal model establishment and administration

All animal experimental protocols and procedures were reviewed and approved by the Animal Ethics Committee of Zhejiang Chinese Medical University, with the ethical approval number: 20230828-04. The experiments were conducted in accordance with the national guidelines for the care and use of laboratory animals. Forty-nine specific pathogen-free (SPF) female BALB/c mice (5-7 months old, approximately 20 g body weight) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (license number: SCXK (Hu) 2017-0005). The mice had free access to food and water. After 5 days of acclimatization, the mice were randomly assigned to seven groups, with 7 mice per group: Normal control group (Normal, N), Model group (Model), Regeneration model group (Regeneration, R), GLP low-dose group (GLP-L), GLP medium-dose group (GLP-M), GLP high-dose group (GLP-H), and metformin group (MET). One day before the experiment, the dorsal fur of all mice was shaved. The N group had normal feeding with free access to water; the Model group had normal feeding with free access to water for 7 days, followed by a 72-hour fasting period; the remaining groups fasted for 72 hours with free access to water, after which the treatments were administered: the N and R groups were given saline by gavage; the GLP-L, GLP-M, and GLP-H groups were given GLP by gavage at doses of 10 mg/kg, 30 mg/kg, and 100 mg/kg, respectively; the MET group received 100 mg/kg metformin via intraperitoneal injection and saline by gavage. After 14 days of intervention, the mice were euthanized with high concentration of carbon dioxide, and tissues were collected for the corresponding experiments and cryopreservation.

2.3 General behavior observation

During the experiment, mice were administered the treatments daily and weighed. General behavior, including food and water intake, was observed.

2.4 Measurement of spontaneous activity

Mice’s spontaneous activity was measured using a spontaneous activity monitoring system. Each mouse was placed individually in an activity box, and its activity was automatically recorded by the system. During the experiment, each mouse was given five minutes to adapt to the environment before recording its spontaneous behavior over the next five minutes. The testing was performed in a dark, quiet environment. After each measurement, the equipment was cleaned with 75% ethanol to eliminate

residual odors.

2.5 Grip strength measurement

After the administration period, grip strength was measured using a grip strength meter. The mouse was lifted by the tail and allowed to grasp the front bar of the meter. After the mouse firmly grasped the bar, it was pulled at a constant speed. Three consecutive measurements were taken, and the average value was used for statistical analysis.

2.6 Organ index measurement

Before euthanasia, the mice's body weight (g) was recorded. The thymus and spleen were removed and excess blood was absorbed with filter paper before photographing to record their size. The organs were weighed using an electronic balance (mg), and the thymic and spleen indices were calculated according to Formula 1.

Visceral index = organ weight (mg) / body weight (g) × 100% (Formula 1)

2.7 H&E histopathological observation

The thymus was fixed in 10% formalin for 24 h and embedded in paraffin. Tissue sections with a thickness of 4 μm were prepared for histopathological analysis. Hematoxylin staining for 5 minutes, and eosin staining for 30 seconds (Hematoxylin - Eosin Stain kit, Nanjing jiancheng, Nanjing, China), using Digital pathological section scanner (KFBIO, China) was used to examine and visualize stained sections.

2.8 Fluorescent immunostaining to observe TEC distribution in mouse thymus tissue

To distinguish the subpopulations and distribution of thymic epithelial cells, the tissue sections were placed in EDTA （Zhongshan Golden Bridge Biotechnology, Beijing, China） antigen repair buffer. The treated sections were stained with primary antibody as follows: rabbit anti-mouse cytokeratin 8 (1: 200) antibody and rabbit mABcytokeratin 5 (1: 200) Antibody. Staining was performed using a Four-color multiplex immunofluorescence kit (Record Biotechnology, Shanghai, China). Cell nuclei was labeled with DAPI. All of the images were obtained using Zeiss fluorescence microscope (AXIO SCOPE. A1, Carl Zeiss AG, Germany) and 3D HISTECH (PANNORAMIC SCAN II, Hungary).

2.9 Flow cytometry analysis

After removing the thymus from the mice, half of each thymus was placed in a well, and the original PBS was removed. Five hundred microliters of pre-cooled PBS was added, and the tissue was gently ground using a sterile syringe plunger to prepare a single-cell suspension. The cell suspension was filtered and transferred to a 15 mL EP tube, followed by an additional 500 μL PBS wash. After centrifugation at 2000 rpm for 5 minutes, the supernatant was removed, and the cells were resuspended in 1 mL of PBS. Five hundred microliters of the cell suspension was transferred to a 1.5 mL EP tube and centrifuged again at 2000 rpm for 5 minutes. After removing 400 μL of supernatant, the antibody incubation process was performed in the dark. Flow cytometry antibodies (CD3 (2306072, BD Pharmingen), CD4 (3095781, BD Pharmingen), CD8 (5223517, BD Pharmingen), TCRB (2040773, BD Pharmingen)) were added to the samples, mixed gently, and vortexed. The samples were incubated at 4 ℃ for 30 minutes, followed by washing with 500 μL of pre-cooled PBS and centrifugation at 2000 rpm for 5 minutes. The supernatant was discarded, and the cells were resuspended in 500 μL of PBS and filtered through a 200-mesh filter. Data were collected and analyzed using a flow cytometer.

2.10 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8.0.2 software. According to the data type and experimental design, non paired *t*-test, one-way

ANOVA (Tukey method for post test) or two-way ANOVA (Tukey method for post test) were selected. Experimental results are presented as mean ± standard deviation, with $p < 0.05$ indicating significant differences, $p < 0.01$ indicating highly significant differences, and $p > 0.05$ indicating no significant differences.

3 Results

3.1 General behavior in mice

As shown in Figure 1, the curves depict changes in food and water intake. The fasting groups exhibited a marked increase in food and water intake on the first day after fasting ended, a significant decrease on the second day, varying degrees of increase on the third day, and a trend toward stabilization on the fourth day. From Day 5 of the experiment onward, daily water intake in each group was relatively stable, whereas food intake showed more pronounced fluctuations.

As shown in Figure 2, during the fasting period, body weight in fasted mice decreased markedly and continuously, reaching the lowest point on Day 4; after resumption of normal feeding, body weight rebounded rapidly. The GLP-M group showed the smallest increase in body weight, whereas the GLP-L group showed the largest increase, increasing by 4.88 g and 6.11 g, respectively, with no statistically significant difference.

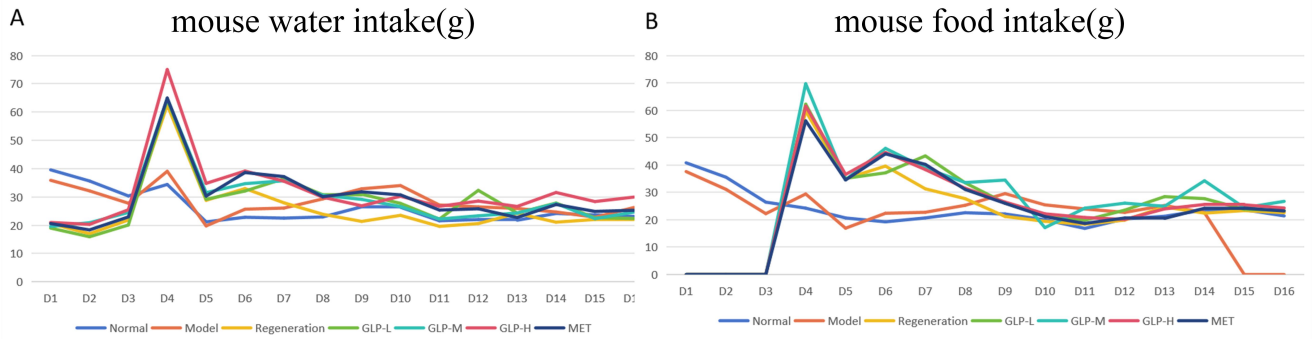

Figure 1 Effects of *Ganoderma lucidum* polysaccharides (GLP) on water intake (A) and food intake (B) in mice (n = 7).

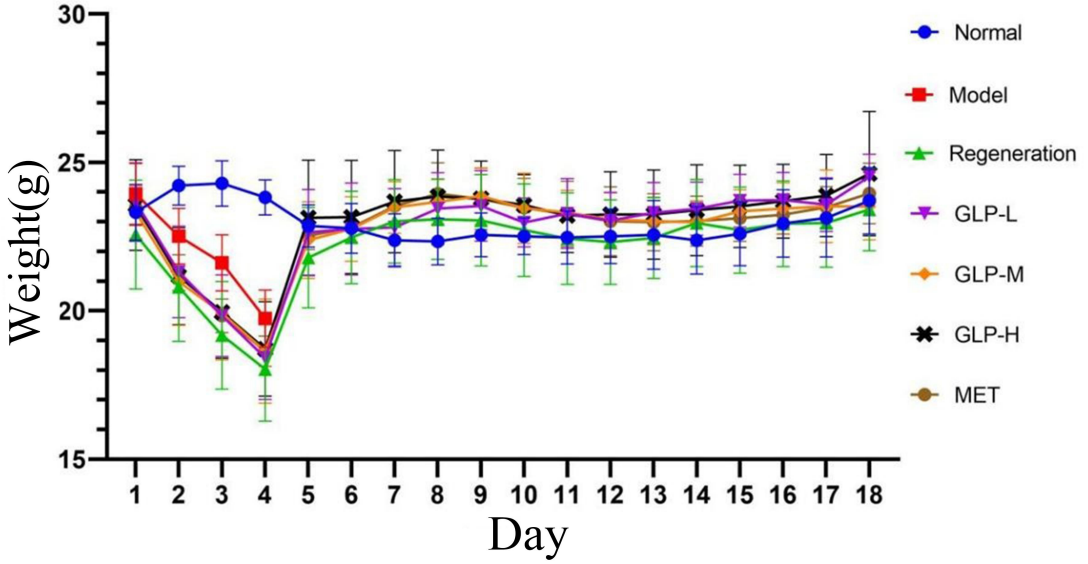

Figure 2 Effect of *Ganoderma lucidum* polysaccharides on body weight in each group of mice (n = 7).

3.2 Spontaneous activity in mice

As shown in Figure 3, the spontaneous activity of mice in the natural regeneration group and the Model group (95.57 ± 21.73) increased compared to the Normal group (86.71 ± 5.56). Notably, the Model group

exhibited a larger standard deviation in spontaneous activity, indicating that the spontaneous activity of mice after starvation fasting was unstable, with individual variability. The GLP-H group showed the most significant increase (109.71 ± 13.49), and there were no statistically significant differences between the groups.

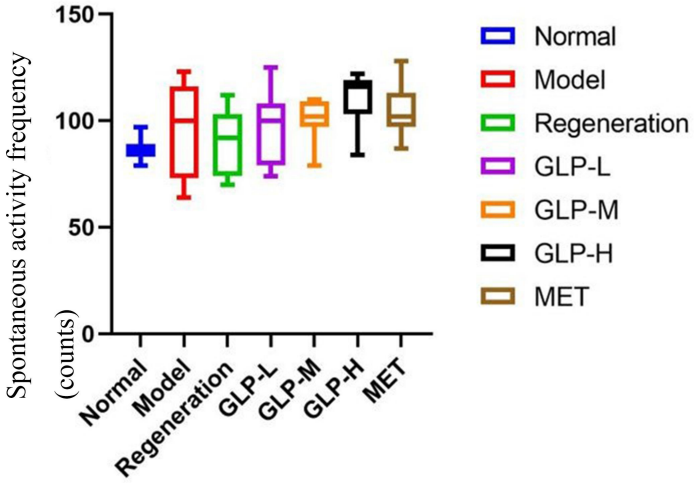

Figure 3 Effect of *Ganoderma lucidum* polysaccharides on spontaneous activity in each group of mice (n = 7).

3.3 Grip strength in mice

As shown in Figure 4, compared to the Normal group, the natural regeneration group showed a slight decrease in grip strength (198.97 ± 32.09). Compared to the Model group, the GLP-L group showed an increase in grip strength to 220.53 ± 28.12. There were no statistically significant differences between the groups.

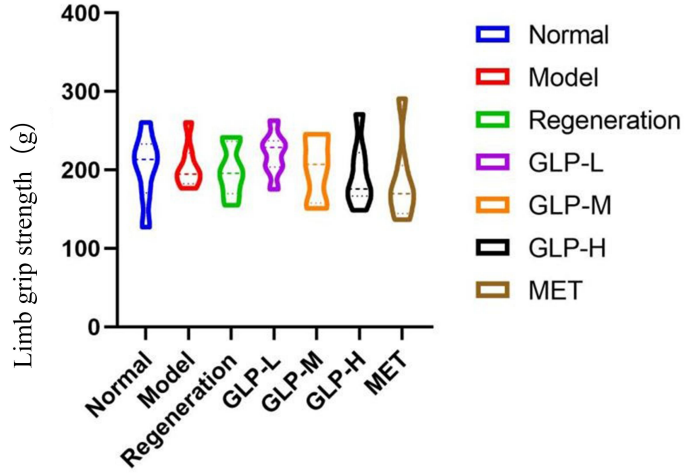

Figure 4 Effect of *Ganoderma lucidum* polysaccharides on limb grip strength in each group of mice (n = 7).

3.4 Organ index in mice

As shown in Figure 5, the thymus and spleen index in the Model group mice significantly decreased ($p < 0.01$). Compared to the Normal group with a thymus index of 0.17 ± 0.01%, the thymus index in the Model group mice dropped markedly to 0.09 ± 0.03%, with a statistically significant difference ($p < 0.001$). Additionally, compared with the Model group, the thymus index in the natural regeneration group was increased. Furthermore, the thymus index of the treatment groups was higher than that of the natural regeneration group, with the GLP-L group showing the greatest increase (0.19 ± 0.03). In addition,

compared to the Normal group, the Model group mice had significant spleen atrophy (spleen index decreased from 0.46 ± 0.07 % to 0.33 ± 0.06 %, $p < 0.01$), whereas the natural regeneration group showed the greatest increase in spleen index.

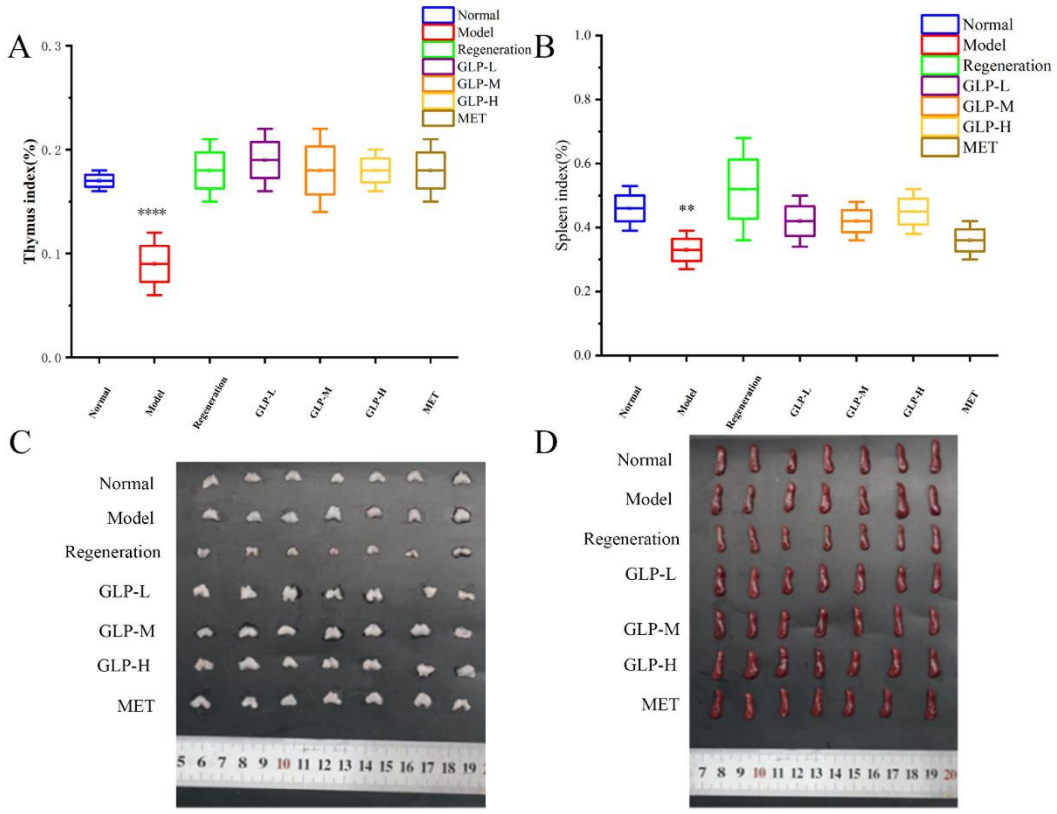

Figure 5 Effects of *Ganoderma lucidum* polysaccharides on thymus and spleen indexes in mice. (A) Effect of *Ganoderma lucidum* polysaccharides on thymic index in each group of mice (n = 7). (B) Effect of *Ganoderma lucidum* polysaccharides on spleen index in each group of mice (n = 7). (C) Representative thymus organs from each group of mice. (D) Representative spleen organs from each group of mice. Compared to the Normal group: ** $p < 0.01$, *** $p < 0.001$.

3.5 H&E staining observations on the effect of *Ganoderma lucidum* Polysaccharides on thymic tissue structure in mice

As shown in Figure 6, the thymus tissue of normal mice exhibited a clear corticomedullary boundary with well-organized cellular arrangement. In contrast, the thymus tissue of Model group mice showed significant atrophy compared to the Normal group, with especially severe cortical atrophy, indistinguishable corticomedullary boundary, and disorganized thymic cells. In the regeneration group, compared to the Model group, the corticomedullary boundary was relatively clear, and the cortical regeneration occurred rapidly, but the medulla remained scattered and not fully connected, suggesting that the thymus could naturally recover after starvation; however, after two weeks, it had not fully returned to normal levels. Compared to the Model group, the treatment groups showed clearer corticomedullary boundaries, with recovery of the corticomedullary structure occurring significantly faster than in the natural regeneration group. Among them, the GLP-H and MET groups exhibited thymic structure most closely resembling the Normal group, indicating that high-dose *Ganoderma lucidum* polysaccharides and metformin most effectively promoted thymic regeneration, resulting in optimal restoration of thymic structure.

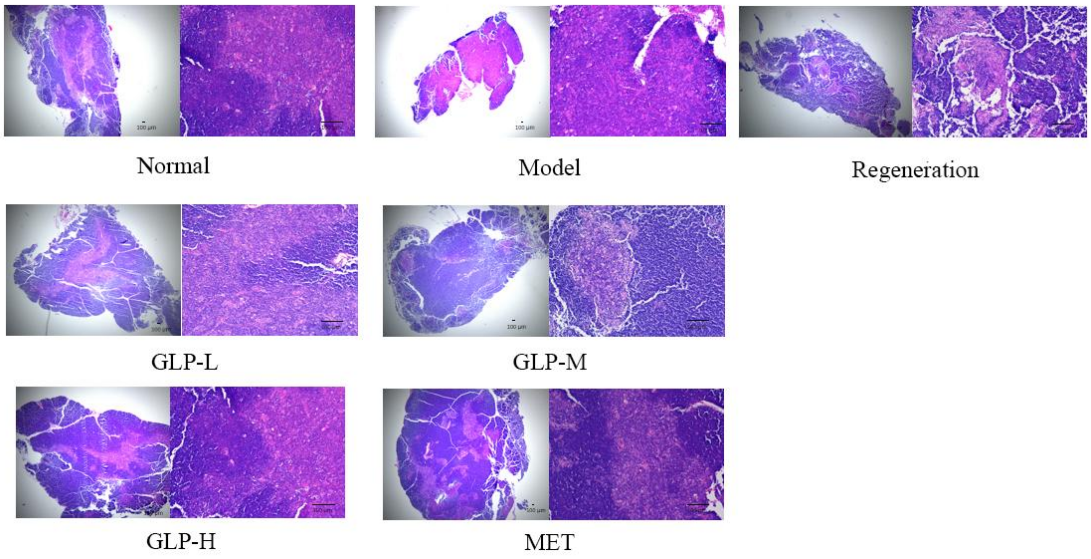

Figure 6 Effect of *Ganoderma lucidum* polysaccharides on thymic structure in each group of mice. Magnification: 4×, 20×.

3.6 Immunofluorescence observation of TECs distribution in mouse thymus

As shown in Figure 7, compared to the Normal group, the reticular structure of the thymus in both the natural regeneration and Model groups showed atrophy. Compared to the natural regeneration group, the GLP-L, GLP-H, and MET groups exhibited a greater proportion of thymic medulla, with increased expression of cytokeratin 5 (CK5) and cytokeratin 8 (CK8) positive cells, indicating a tighter cortical epithelial network. This suggests that *Ganoderma lucidum* polysaccharides and MET have a certain role in thymic function recovery.

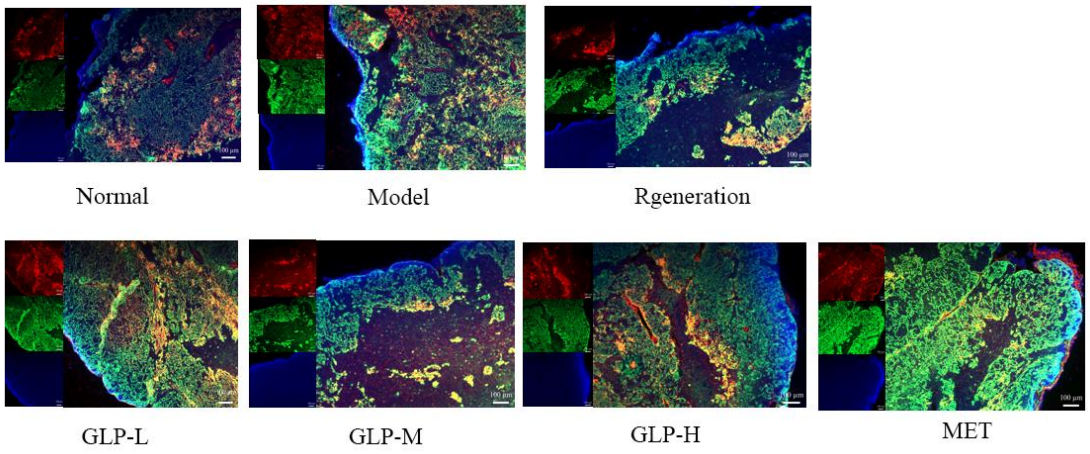

Figure 7 Effect of *Ganoderma lucidum* polysaccharides on the spatial distribution of TECs in the thymus of each group of mice. Magnification: 10×, CK5 (red)/CK8 (green)/DAPI (blue).

3.7 Effect of *Ganoderma lucidum* Polysaccharides on T-Cell subset differentiation in mouse thymus

As shown in Figure 8, compared to the Normal group, the percentages of $CD8^+$SP cells and $CD4^+$SP cells increased in Model group, while the percentage of DP T cells significantly decreased to 63.31%. This may be due to fasting preventing the conversion of DN T cells

into DP T cells. After resumption of feeding, the percentages of DN T cells, $CD8^+$SP cells, and $CD4^+$SP cells decreased, while the percentage of DP T cells increased. Compared to the Regeneration group, the percentages of DN T cells increased in GLP-H group, while the percentage of DP T cells significantly decreased to 63.34%. The proportion of all cells, including DP, DN, $CD8^+$SP cells, and $CD4^+$SP cells, were closer to the Normal group.

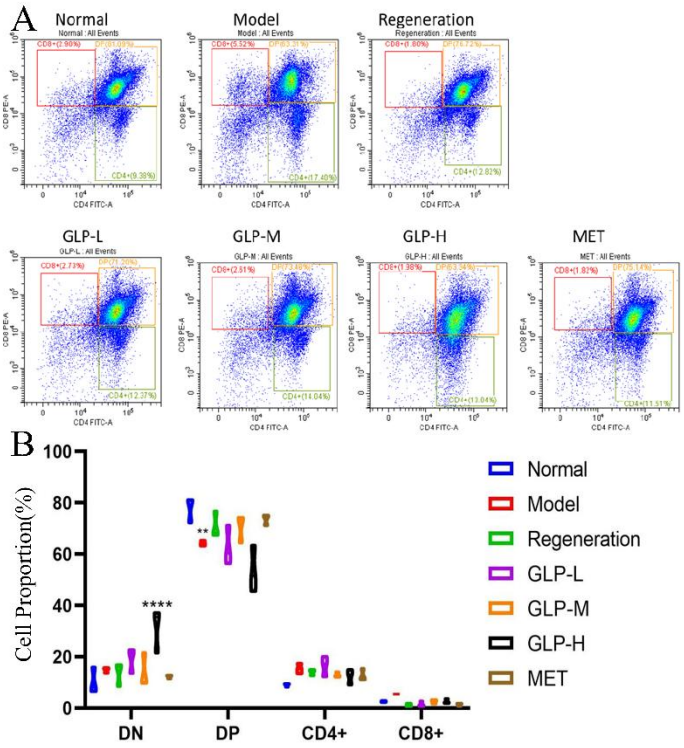

Figure 8 Effect of *Ganoderma lucidum* polysaccharides on the proportion of T-cell subsets in the thymus of each group of mice (n = 3). Compared to the Regeneration group: **** $p < 0.001$, ** $p < 0.01$.

4 Discussion

In this study, we established a 72-hour acute fasting mouse model to simulate the impact of severe energy deprivation on thymic structure and function and to further evaluate the potential promotive effects of *Ganoderma lucidum* polysaccharides (GLP) on thymic regeneration. The results showed that fasting significantly reduced the thymus index in mice (from 0.17 ± 0.01% to 0.09 ± 0.03%), indicating that energy restriction rapidly induces thymic atrophy. Although ad libitum refeeding restored the thymus index to 0.18 ± 0.03%, cortical-medullary architecture and T-cell subset distribution were not fully recovered, indicating the limitations of spontaneous regeneration. Compared with the spontaneous regeneration group, the GLP-treated group, particularly at medium and high doses, exhibited more clearly defined cortical-medullary boundaries. Immunofluorescence revealed a marked increase in CK5- and CK8-positive thymic epithelial cells (TECs) and reticular network density, and flow cytometry demonstrated elevated proportions of double-negative (DN) T cells and $CD8^+$ single-positive (SP) cells. These findings suggest that GLP may promote the restoration of thymic structure and function following acute injury by improving the thymic microenvironment, enhancing TEC activity, and modulating T-cell differentiation.

Recent studies have extensively investigated the immunomodulatory and cytoprotective effects of GLP. Li et al. reported that GLP supplementation significantly activates T cell-mediated antitumor

immunity and enhances the efficacy of anti-PD-1 immunotherapy, indicating that GLP can regulate adaptive T-cell function and enhance immune responses, which is consistent with our observation of increased T-cell subset proportions following GLP treatment [9]. Additionally, review studies have highlighted that GLP possesses anti-inflammatory, antioxidant, and immunoregulatory properties, capable of modulating the function of immune cells such as macrophages, T cells, and B cells to improve systemic immune status [5]. GLP has also been shown to modulate gut microbiota and immune cell interactions, providing protective effects in models of inflammation and immune-related diseases, which underscores its potential to regulate the immune microenvironment [10]. Collectively, these studies support the multi-layered immunomodulatory and tissue-protective roles of GLP, consistent with the thymic regenerative effects observed in our study.

Mechanistically, the thymus is a critical organ for T-cell differentiation, and its microenvironment and TECs are essential for maintaining thymocyte lineage development. Malnutrition or energy restriction can impair the thymic microenvironment by downregulating metabolic signaling and altering hormonal levels, leading to a reduction in cortical lymphocytes and TEC dysfunction, thereby affecting DN, double-positive (DP), and other thymocyte subsets and ultimately suppressing the generation of mature SP T cells [11]. In our study, GLP significantly increased the proportions of DN and $CD8^+$SP cells, suggesting that it may indirectly promote precursor T-cell proliferation and differentiation by improving the thymic microenvironment. Furthermore, GLP may support thymocyte survival and proliferation by enhancing local cytokine expression and mitigating oxidative stress. Reviews emphasize that microenvironmental stability is critical for endogenous thymic regeneration, and its improvement can enhance regenerative efficiency [11]. Although direct evidence remains limited, it is reasonable to speculate that GLP facilitates thymic repair and regeneration by improving local metabolism and maintaining microenvironmental homeostasis.

Acute energy restriction not only affects thymic structure but may also induce metabolic stasis, thereby inhibiting thymocyte proliferation and immune activity. Fasting reduces ATP generation, and insufficient energy supply can result in cell cycle arrest or apoptosis, exacerbating thymic involution. GLP has been demonstrated to possess antioxidant and immunomodulatory properties, which may help cells mitigate oxidative damage and maintain basic metabolism and differentiation under energy-limited conditions, partially reversing fasting-induced thymic suppression. Some foundational studies have suggested that immunomodulatory natural compounds can improve gut-immune interactions and influence inflammatory and metabolic signaling, a mechanism that may also extend to the thymic microenvironment [10]. Therefore, GLP may enhance thymic energy supply and signaling homeostasis through multiple pathways, promoting structural and functional recovery.

Thymic epithelial cells (TECs) are core components for maintaining the thymic microenvironment and supporting positive and negative selection of T cells. Improvement in TEC number and function is critical for thymic tissue recovery. Studies have shown that reductions in TECs during aging or after injury are closely associated with thymic functional decline, while improvement of the TEC microenvironment contributes to restoring thymic output and immune function [11]. In our study, immunofluorescence results demonstrated that GLP increased the proportion of CK5- and CK8-positive TECs and reticular network density, suggesting that GLP may promote TEC proliferation or inhibit apoptosis, thereby enhancing thymic support for T-cell development.

Similar mechanisms have been observed for other natural polysaccharides and herbal compounds, which improve organ microenvironments to facilitate tissue regeneration, providing a rational basis for GLP's effects.

In conclusion, this study systematically evaluated the effects of GLP in a model of acute fasting-induced thymic involution. The results indicate that GLP significantly improves thymic structure and function, including enhancing TEC microenvironment, increasing key T-cell subset proportions, and promoting tissue reconstruction. These findings extend the known immunomodulatory roles of GLP and provide both theoretical and experimental support for using natural compounds to promote immune organ regeneration. Future studies should further elucidate the molecular mechanisms by which GLP regulates thymic metabolism, epithelial cell signaling pathways, and T-cell differentiation, advancing its potential applications in immune recovery and interventions for immune-compromised states.

Acknowledgements

Not applicable.

Conflicts of Interest

The author of this article, Jianli Gao, is the editor-in-chief of the editorial office of this journal. All procedures during the editorial review process were conducted strictly in accordance with the journal's policies, and the author was not involved in handling any part of the process.

Author Contributions

X.L.: Writing-original draft, Conceptualization, Methodology. Y.X.: Writing-review, Data curation. J.G.: Writing-review and editing, Visualization, Funding acquisition, Project administration, Resources, Supervision. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. X.L. and Y.X. contributed equally to this work and shared the first authorship.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Animal Ethics and Welfare Committee of Zhejiang Chinese Medical University (Approval No.: IACUC-20230828-04) and strictly followed the guidelines certified by AAALAC during the experimental process, ensuring that the care and use of experimental animals complied with ethical and scientific standards.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 82474614).

Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary

Not applicable.

References

[1] Duah M, Li L, Shen J, et al. Thymus degeneration and regeneration. *Frontiers in Immunology* 2021; 12: 706244.

[2] Li YR, Zúñiga-Pflücker JC. Thymus aging and immune reconstitution. *Seminars in Immunology* 2023; 70: 101837.

[3] Zhao L, Geng Z, Wang Y, et al. Immunomodulatory effects of Ganoderma lucidum bioactive components. *Current Issues in Molecular Biology* 2025; 47(10):842

[4] Iser-Bem PN, Lobato TB, Alecrim-Zeza AL, et al. Ganoderma lucidum dry extract supplementation modulates T lymphocyte function in older women. *The British Journal of Nutrition* 2024,132(2): 130-140.

[5] Ren L, Zhang J, Zhang T. Immunomodulatory activities of polysaccharides from Ganoderma on immune effector cells. *Food Chemistry* 2021; 340: 127933.

[6] Guo C, Guo D, Fang L, et al. Ganoderma lucidum polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon.

Carbohydrate polymers 2021; 267: 118231.

[7] Han XQ, Yue GL, Yue RQ, et al. Structure elucidation and immunomodulatory activity of a beta glucan from the fruiting bodies of Ganoderma sinense. *PLoS One* 2014; 9(7): e100380.

[8] Liu KS, Zhang C, Dong HL, et al. GSP-2, a polysaccharide extracted from Ganoderma sinense, is a novel toll-like receptor 4 agonist. *PloS one* 2019; 14(8): e0221636.

[9] Li W, Zhou Q, Lv B, et al. Ganoderma lucidum polysaccharide supplementation significantly activates T-Cell - mediated antitumor immunity and enhances anti - PD - 1 immunotherapy efficacy in colorectal cancer. *Journal of Agricultural and Food Chemistry* 2024; 72(21): 12072 - 12082.

[10] Chen ZC, Zhang HJ. The interaction between Ganoderma lucidum polysaccharides and gut microbiota: implications for immune health. *Medicinal Plant Research* 2025; 15(1): 1-11.

[11] Savino W, Durães J, Maldonado - Galdeano C, et al. Thymus, undernutrition, and infection: approaching cellular and molecular interactions. *Frontiers in Nutrition* 2022; 9: 948488.