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Fabricius Extract Effect on the Morphofunctional State of the Thymus and Spleen in Cyclophosphamide-Induced Mice

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ABSTRACT

This article examines the effects of the bursa of Fabricius extract on the morphofunctional state of the spleen and thymus in mice with cyclophosphamide-induced immunodeficiency. Histological analysis of the spleen revealed that in immunodeficient mice, the white pulp is characterized by a diffuse arrangement of lymphoid follicles without germinal centers. Administration of the bursa of Fabricius extract resulted in a decrease in the area of red pulp and reactive centers in the spleen, but signs of extramedullary hematopoiesis were observed. Immunohistochemical analysis revealed an increase in the number of CD3+ + T lymphocytes in the spleen after administration of the extract, indicating an enhanced T-cell immune response and suppression of immunosuppression. No significant changes in the structure or cellular composition of the thymus were observed under the influence of the extract. The data indicate that Bursa Fabricius extract has an immunomodulatory impact, especially affecting the spleen.. This is manifested by stimulation of the proliferation and differentiation of T- and B-lymphocytes, as evidenced by an increase in the number of T-lymphocytes in the spleen. It is believed that this effect is mediated through specific receptor-ligand interactions in the organs of immunogenesis. Thus, the study demonstrates the potential use of Bursa Fabricius extract for the correction of immunodeficiency conditions by acting on the spleen.

Keywords: *Bursa of Fabricius extract, Spleen, Thymus, Immunomodulation, T-lymphocytes, Morphofunctional state*

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INTRODUCTION

The primary sources of raw materials used in the pharmaceutical industry for veterinary and medical purposes as immunomodulatory biologically active substances include the placenta, lymph nodes, thymus, spleen, and bone marrow (Shirani *et al*., 2015; Kolberg, 2017; Ariposvky & Titov, 2019). All tissue used as raw materials is obtained from piglets and calves, with the placenta sourced from pregnant cows (Kolberg, 2015; Zhu *et al*., 2022; Sun *et al*., 2024). These organs play a crucial role in the formation of innate and acquired immunity, humoral and cellular immunity, as well as in hemocytopoiesis (Wieczorek *et al*., 2022; Zemskov *et al*., 2022; Santos *et al*., 2024). The bone marrow, spleen, and thymus are central organs of the immune system, while lymph nodes are considered peripheral organs (Arkhincheeva & Balkhaev, 2022; Ridley *et al*., 2024; Raja *et al*., 2025).

These organs contain various cell types that play a key role in immune responses, including T cells, B cells, and macrophages (Sanchez-Ramon *et al*., 2016; Sánchez-Ramón *et al*., 2019; Rao Muvva *et al*., 2021). The use of tissue from these organs in the pharmaceutical industry allows for the development of drugs that can enhance or modulate the body's immune response (Park & Kim, 2025; Sharafshah *et al*., 2025; Tan *et al*., 2025). This is particularly important in veterinary medicine for the prevention and treatment of infectious diseases (Pescovitz *et al*., 2011; Zhang *et al*., 2018; Song *et al*., 2021; Yun *et al*., 2022; Berglund *et al*., 2023; Kolberg *et al*., 2023; Tabolacci *et al*., 2023; Zavdy *et al*., 2023; Purohit *et al*., 2024; Tan *et al*., 2025; Yun *et al*., 2025).

MATERIALS AND METHODS

Immunohistochemical staining was utilized to detect and study cell populations in the thymus and spleen of laboratory mice. The study identified the presence of CD45+ cells, a marker for all leukocytes, as well as specific B- and T-lymphocyte antigens: CD20 and CD3, respectively (Roberts *et al*.,2015). Monoclonal antibodies, as listed in **Table 1**, were used for the study. All reagents used were adapted for use with paraffin sections and complied with the manufacturers' recommendations.

Table 1. List of antibodies used in the study

Antigen	Primary Antibody: Clone, Dilution, Manufacturer	Secondary Antibody: Clone, Dilution, Manufacturer
CD 45	CD45 Polyclonal Antibody, 1:100, PA5-96061, Invitrogen, Thermo Fisher Scientific, USA.	Goat anti-Rabbit IgG (H+L) Secondary Antibody, Biotin, polyclonal, 1:1000, 65-6140, Thermo Fisher Scientific, USA.
CD20	CD20 Polyclonal Antibody, 1:300, PA5-16701, Invitrogen, Thermo Fisher Scientific, USA.	
CD3	Anti-CD3, T Cell antibody produced in rabbit, polyclonal, 1:200, C7930, Sigma-Aldrich, Merck, USA	

To prepare tissue sections for immunohistochemical analysis, glass slides coated with Snowcoat X-tra adhesive (Leica Biosystems, USA) were used to ensure reliable specimen retention during multi-step processing. After cutting paraffin blocks with a microtome, sections were placed on the slides and allowed to dry at room temperature. Deparaffinization was then carried out stepwise using xylene and a series of rehydration washes in decreasing alcohol concentrations. This process effectively removed paraffin and restored the necessary tissue hydration level for subsequent immunohistochemical staining. Before staining, the sections were rinsed in PBS-Tween 20 buffer (pH 7.2–7.4) to eliminate residual contaminants and create optimal conditions for subsequent reactions.

After tissue fixation with formalin, antigen epitopes may become less accessible for antibody binding due to the formation of additional cross-links between protein molecules. To restore antigen accessibility, a retrieval procedure is used. Following the technical protocol recommended by the manufacturer of CD3 antibodies, sections were pretreated with trypsin. A 0.05% trypsin working solution (Sigma-Aldrich, Inc.) was prepared using distilled water with the addition of a 1% calcium chloride solution. The tissues were heated to 37°C in a thermostated water bath in order to activate the enzyme. After applying the trypsin solution to the prepared sections, they were kept at the same temperature for 15 minutes in a humid room..

After processing, the tissue slides were rinsed twice with cold distilled water to effectively stop the enzyme action. The sections were then rinsed again in a PBS solution supplemented with Tween 20 (PBS-Tween 20).

All subsequent immunohistochemical analysis procedures were performed in a uniform manner, applicable to all target antigens. Antigen expression was visualized using indirect peroxidase staining. Sections were incubated with primary antibodies for 60 minutes at 37°C in a humidified chamber, then washed twice in PBS-Tween 20.

To block the activity of endogenous peroxidase, a ready-made Peroxidase reagent was used (Novocastra, UK). After this, the sections were washed twice again with the same buffer.

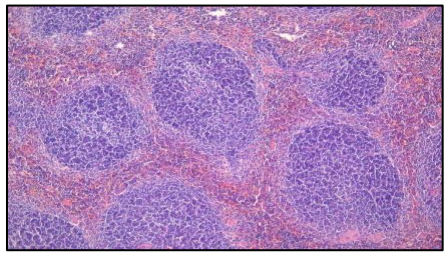
The next step involved a 30-minute incubation with secondary antibodies at 37°C under high humidity. Following this, the slides were washed twice with PBS-Tween 20. Detection of antigen-antibody complexes was performed using the Novolink detection system. Polymer Detection System (Novocastra, UK).

The final visualization was performed using the chromogen DAB (3,3-diaminobenzidine), which stained the cell cytoplasm an intense brown hue in the presence of specific expression.

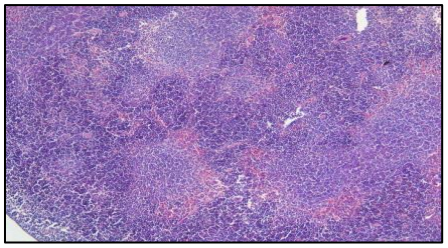
Positive controls were established based on known antigen-positive tissue areas, while negative controls included replacing the primary antibody with a non-specific isotype control or omitting it on parallel sections of the samples being studied.

RESULTS AND DISCUSSION

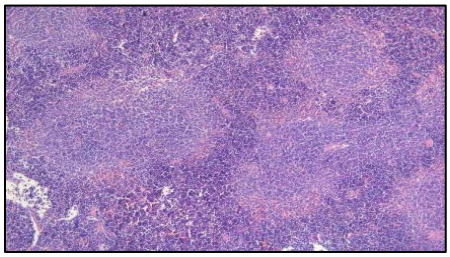
Upon histological analysis of spleen sections from mice with immunodeficiency induced by cyclophosphamide, it is evident that in induced animals, the white pulp consists of diffusely distributed lymphoid follicles lacking light centers with central arterioles and periarteriolar lymphocyte clusters **(Figure 1)**. The overall cellularity of the organ remains unchanged.



a)



b)



c)

Figure 1. Spleens of C57BL/6 mice. a) intact mice; b) mice with cyclophosphamide-induced immunodeficiency, and c) mice with cyclophosphamide-induced immunodeficiency who were also administered bursa of Fabricius extract. The samples were stained with hematoxylin and eosin. A 10x magnification was used for the analysis.

Tables 2 and 3 provide characteristics of volumetric ratios of red and white pulp of the spleen in mice from the experimental groups and morphometric parameters of the zones of lymphoid nodules in the spleen.

Table 2. Characteristics of volumetric ratios of the red and white pulp of the spleen in mice from the experimental groups

Group	Size (mm^2)	S of the red pulp (mm^2)	S of the white pulp /S of the red pulp	Number of lymphoid follicles/1 mm^2 of parenchyma
CFA	1.272±0.095	5.502±0.203	0.305±0.029	2.087±0.119
CFA + Bursantal	1.454±0.097	6.469±0.082*	0.268±0.025	2.429±0.167

* - significant differences with the intact group indicator ($p < 0.05$).

Table 3. Morphometric parameters of the zones within lymphoid nodules of the spleen

Group	S reactive center (μm ^{2})	S mantle zone (μm^2)	S of the marginal zone (μm ^{2})
CFA	8494±1533	44481±4692	21797±2382
CFA + Bursantal	6477±333*	56449±2683	24769±3912

* - significant differences with the intact group indicator ($p < 0.05$).

When studying the morphofunctional state of the spleen in cyclophosphamide-induced animals with the introduction of bursa of Fabricius extract, a reliable decrease in the area of the red pulp and a decrease in the area of the reactive center were observed **(Table 4)**. However, areas of extramedullary haematopoiesis are diffusely determined in the stroma as megakaryocytic series colony-forming cells..

Immunohistochemical determination of the pan-leukocyte marker on red and white pulp cells revealed no changes in $CD45^+$ counts. However, $CD3^+$ counts were significantly elevated, a characteristic sign of suppressed immunosuppression and enhanced T-cell responses (Poornachitra & Maheswari, 2023; Yurievna *et al*., 2023).

Table 4. The area of the medulla and cortex of the thymus

Group	S of medulla (mm^2)	S of cortical substance (mm^2)	S of medulla / S of cortex
CFA	0.613±0.127	2.702±0.583	0.382±0.185
CFA + Bursantal	0.637±0.123	3.516±0.523	0.277±0.015

Morphometric analysis of the thymus in mice with induced immunodeficiency showed that the use of bursa of Fabricius extract did not result in consistent changes in the area of the cortex and medulla layers of the organ compared to mice treated with cyclophosphamide (CPA) alone. Immunohistochemical staining revealed no statistically significant differences in the quantitative distribution of CD45+ and CD3+-positive cell populations in the structural zones of the thymus. Quantitative analysis demonstrated the preservation of a standard cellular composition in both the cortical and medullary regions of the organ when calculated per 1 mm^2 of the examined tissue **(Table 5)**. These findings suggest that the bursa of Fabricius extract does not impact the cellular composition and structural organization of the thymus in mice with CFA-induced immunodeficiency (Botelho *et al*., 2023; Bulusu & Cleary, 2023).

Table 5. Distribution density of $CD45^+$ and $CD3^+$ $^+$ cells in the medulla and cortex of the thymus in C57BL/6 mice

Group	$CD45^+$ cells in 1 mm^2 of cortex	CD45+ cells in 1 mm^2 of medulla	$CD3^+$ cells in 1 mm^2 of the cortex	$CD3^+$ cells in 1 mm^2 of cortical substances
CFA	112505 ±8524	87594 ±2114	5312.85±738.59	6315.92±1504.54
CFA + Bursanatal	1 29979 ±6990	89468 ±1589	6267.15±790.94	11648.77±2263.73

CONCLUSION

It can be hypothesized that the extract from the bursa of Fabricius in birds, when administered to laboratory mice with experimental immunodeficiency, likely due to specific receptor-ligand regulation, exhibits an affinity for immunogenesis organs, primarily the spleen. This demonstrates pronounced immunomodulatory properties, presumably consisting of enhanced proliferation and differentiation of T- and B-lymphocytes. This conclusion is supported by a sharp increase in the number of T+ lymphocytes in both the white and red pulp of the spleen.

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ETHICS STATEMENT: The study was conducted according to the guidelines of the Declaration of Helsinki.

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