

# Effectiveness of Pomegranate Seed Oil in the Correction of Morphological Changes in Thymic Tissue During Experimental Chemotherapy

Rasulova N. R.\* , Temirova N. R.

Bukhara State Medical Institute, Uzbekistan

**Abstract** During chemotherapy, cytostatic drugs exert a significant toxic effect on the thymic tissue, which is one of the main organs of the immune system. As a result, a decrease in lymphoid elements, cortical zone atrophy, dystrophic changes in the medullary areas, as well as a relative increase in stromal components, are observed. These alterations lead to a decline in immunological defense mechanisms, increasing the susceptibility of the organism to various infectious and inflammatory processes. In recent years, the use of natural antioxidants and immunomodulatory agents has been considered one of the important approaches to reducing adverse effect of chemotherapy. In this study, morphological and morphometric changes in thymic tissue caused by chemotherapy in experimental animals were investigated, and the possibilities of their correction using pomegranate seed oil were evaluated. The results demonstrated that in the groups treated with pomegranate seed oil, the thymic cortical layer was relatively better preserved, the activity of the germinal centers of the follicles was restored, and the compensatory remodeling of stromal components approached physiological norms.

**Keywords** Chemotherapy, Thymus, Pomegranate seed oil, Immunocorrection, Morphometry, Antioxidant, Immunomodulator

## 1. Introduction

Today, oncological diseases are among the most pressing health problems worldwide, causing high morbidity and mortality rates. One of the main methods used in the treatment of malignant tumors is chemotherapy. Cytostatic drugs inhibit the division of tumor cells and lead to their destruction. [1] However, these drugs also negatively affect normal tissues, especially organs with rapidly dividing cells, such as bone marrow, intestinal epithelium, and thymus. The thymus, as the central organ of the immune system, ensures T-lymphocyte differentiation and immune surveillance. During chemotherapy, degenerative changes in lymphoid cells, significant narrowing of the cortical layer, decreased activity of germinal centers in follicles, and relative proliferation of stromal elements occur in thymic tissue. These morphological changes weaken the patient's immunobiological stability and create conditions for the development of secondary infections, autoimmune processes, and relapses. Therefore, supporting the immune system during and after chemotherapy is both a relevant scientific and practical issue. [2]

In recent years, scientific research has focused on methods of protecting and restoring the immune system using biologically active substances of natural origin. Pomegranate seed oil (*Punica granatum L.*) contains a high amount of polyphenols, flavonoids, tocopherols, punicic acid, and other unsaturated fatty acids. Their strong antioxidant, anti-inflammatory, and cytoprotective properties have been scientifically proven. For this reason, pomegranate seed oil may reduce oxidative stress, slow down the process of apoptosis in immune cells, and stimulate the regeneration of lymphoid tissues in the thymus.

Thus, the issue of correcting chemotherapy-induced morphological changes in thymic tissue with the help of pomegranate seed oil is relevant not only for fundamental morphology but also for practical medicine. The results of this study provide a theoretical basis for the use of natural antioxidants in alleviating the adverse effects of chemotherapy and may, in the future, allow their broad application in clinical practice as an adjunct therapy to support the immune system.

**Purpose of the study.** To identify morphological and morphometric changes occurring in thymic tissue under experimental chemotherapy conditions and to evaluate the effectiveness of their correction using pomegranate seed oil.

## 2. Materials and Methods

This research was conducted at the Scientific Research

\* Corresponding author:

nafisarasulova1989@gmail.com (Rasulova N. R.)

Received: Aug. 25, 2025; Accepted: Sep. 17, 2025; Published: Dec. 31, 2025

Published online at <http://journal.sapub.org/ajmms>

Laboratory of Bukhara State Medical Institute named after Abu Ali ibn Sina. The experimental stages were organized in accordance with a pre-developed plan based on the requirements of research methodology. Particular attention was paid to ensuring a stepwise approach, adherence to bioethical standards, and achieving sufficient statistical sample size to obtain reliable results.

#### Experimental Design

The experimental work was based on the principles of empirical medicine. At the initial stage, laboratory animals (white outbred rats) were bred and randomly divided into groups. The groups were formed as control and experimental groups, which helped ensure the representativeness and reliability of observations. During the preparation phase of the experiment, feeding, testing conditions, and necessary factors were standardized.

#### Experimental Groups

Three main groups were formed within the framework of the study:

1. Control group (n=10): Healthy white outbred rats used for comparison with the experimental groups.
2. Experimental group 1 (n=10): Rats in which mammary gland carcinoma was induced using 7,12-dimethylbenz[a]anthracene (DMBA). The obtained results were confirmed by the CA-15-3 (Cancer Antigen 15-3) tumor marker.
3. Experimental group 2 (n=10): Rats with mammary gland carcinoma that were administered paclitaxel at a dose of 0.4 mg/kg intravenously and a dose of 0.2 mg/kg and 0.7 ml of pomegranate seed oil intragastrically via a stomach metal probe for 21 days.

#### Collection of Biomaterial

At the end of the experimental period, the animals were sacrificed by decapitation. The abdominal cavity was opened, and the thymus glands were excised. From each thymus, tissue fragments measuring approximately  $5 \times 3 \times 3$  mm were cut and prepared for histological examination. The samples were fixed in 10% neutral formalin, ensuring that the volume of fixative exceeded the tissue volume by at least 20–25 times.

#### Histological Processing

After fixation, the material was dehydrated in a graded series of ethanol (from 40° to 96°). To ensure the quality of dehydration, the method described by Yanin V.L. et al. (2015) was used. Following dehydration, the material was embedded in paraffin blocks. For optimal sectioning, 5 g of wax was added to 100 g of paraffin.

Sections with a thickness of 5–7 nm were prepared and stained with histological dyes. Ehrlich's hematoxylin was used as the primary stain, and eosin as the counterstain. Additionally, the Van Gieson staining method was applied to visualize stromal and connective tissue elements. The stained preparations were mounted with Canada balsam. Prepared micropreparations were examined under an

NLCD-307B light microscope, and photomicrographs were taken. Morphometric parameters (thickness of the thymic capsule, ratio of cortical and medullary zones, follicle diameter, etc.) were calculated using specialized software.

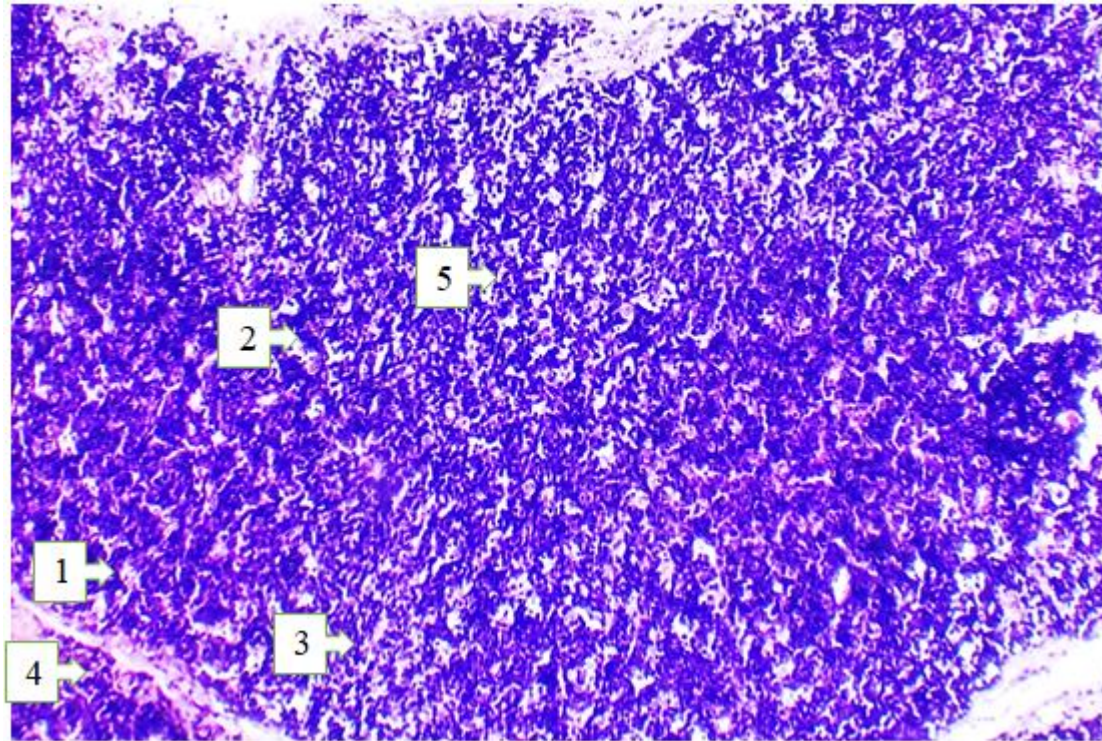
All stages of the study were pre-approved by the Ethics Committee, and all procedures were carried out in accordance with biosafety regulations. The results were recorded under the supervision of a scientific advisor and subjected to statistical analysis at the final stage. [3]

### 3. Results and Discussions

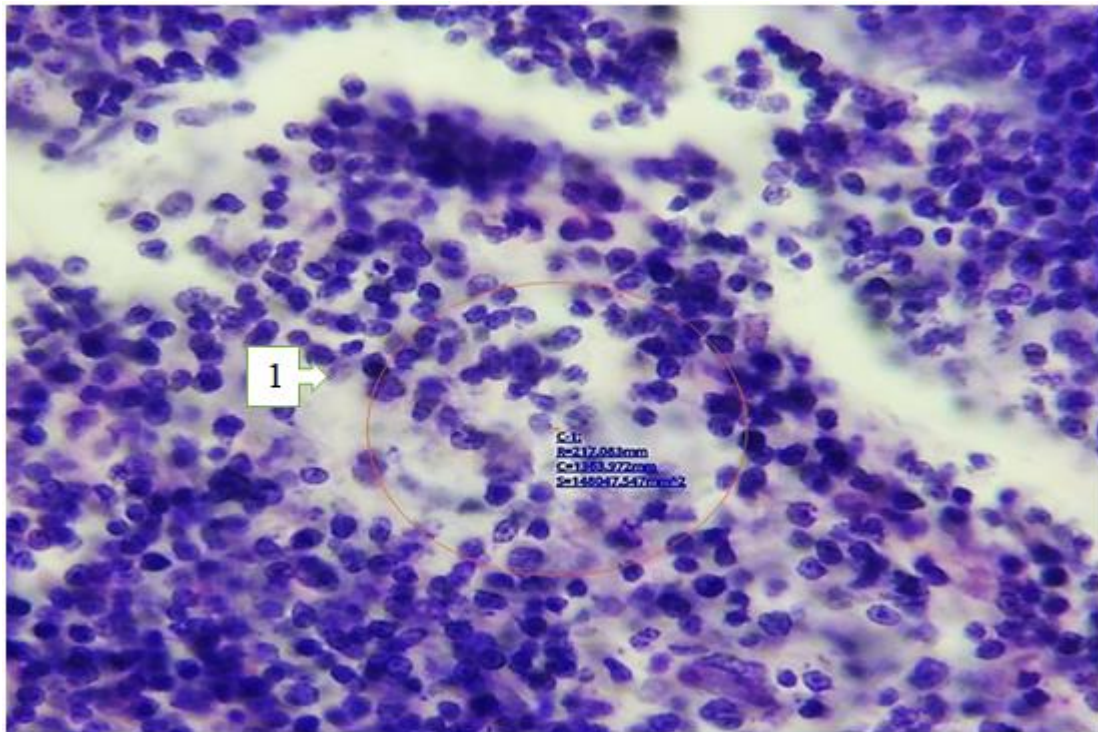
When studying the morphological changes in thymic tissue under the influence of chemotherapeutic drugs using hematoxylin–eosin staining, the following alterations were most prominent. Under the effect of chemotherapeutic agents, a reduction in thymic size was observed, along with fatty changes in the surrounding tissue and slight thickening of the capsule. Examination of histological preparations under higher magnification revealed that the main structural components of the thymic lobules—the cortex and medulla—were stained uniformly, which was the first noticeable feature. This phenomenon indicated that T-lymphocytes in the cortical region had been released into the peripheral blood and dependent regions, resulting in a reduced number of mature T-lymphocytes. At the same time, thickening of the cytotreticulum, i.e., the blood-thymus barrier, reflected impaired blood supply to the tissue. In turn, this condition, combined with the toxic effects of chemotherapeutic agents, led to the early toxic apoptosis of lymphoblasts. [7]

After experimental chemotherapy, in the preserved thymic tissue, the interlobular septa and the parallel cytotreticulum were thickened and expanded, stained light pink, which indicates degenerative changes in proteins within the connective tissue. In the remaining thymocytes, hyperplasia was detected, with enlarged, hypochromatically stained nuclei and eosinophilic cytoplasm, along with an increased number of macrophages. The proliferation and activation of macrophages, resulting from altered function of the blood-thymus barrier, led to impaired development of lymphoblasts, which in turn activated apoptotic mechanisms in thymocytes and increased phagocytosis of defective lymphoblasts.

During the study of thymocyte morphometry in the thymus of white outbred rats under experimental chemotherapy, the thickness of the thymic capsule ranged from  $5.22 \pm 0.4$  nm to  $12.2 \pm 0.5$  nm, with an average of  $9.24 \pm 0.6$  nm; the subcapsular zone thickness ranged from  $3.5 \pm 0.4$  nm to  $6.18 \pm 0.5$  nm, with an average of  $5.89 \pm 0.7$  nm; trabecular thickness ranged from  $2.78 \pm 0.62$  nm to  $6 \pm 0.73$  nm, averaging  $4.54 \pm 0.5$  nm. The interlobular artery measured 17–20 nm (average 18 nm), the venous diameter ranged from 12–28 nm (average 21 nm), and postcapillary venules ranged from 7–11 nm (average 9 nm). The enlargement of blood vessels was associated with thickening of their walls.



**Figure 1.** Microscopic view of the thymus of a 3-month-old white outbred rat after experimental chemotherapy. Hematoxylin and eosin staining. Magnification 10x20. 1 – Decreased thymocytes around reticuloepithelial cells; 2 – Uniform staining of the cortical and medullary regions of the lobules, indicating a reduction in lymphocytes in both zones; 3 – Reduction of T lymphocytes in the cortical region of the lobules as a result of increased apoptosis; 4 – Shrinkage of lobules; 5 – Increased number of macrophages



**Figure 2.** Morphometry of thymocytes in the cortical region of the thymus of a 3-month-old white outbred rat after experimental chemotherapy. Hematoxylin and eosin staining. Magnification 20x20. 1 – Morphometric measurements of T lymphocytes (thymocytes) in the cortical region of the thymus

The number of thymocytes in the cortical and medullary regions of each thymic lobule was also evaluated. In the cortex, thymocyte density ranged from 4 to 9 per  $\text{mm}^2$  with

an average of 7 per  $\text{mm}^2$ ; in the medulla, thymocyte density ranged from 2 to 6 per  $\text{mm}^2$  with an average of 4 per  $\text{mm}^2$ . When calculated per standard visual field, the number of

thymocytes in the cortical region ranged from 76 to 153, averaging  $104 \pm 18$ , whereas in the medullary region it ranged from 44 to 103, averaging  $62 \pm 11$ . The analyses demonstrated that the number of thymocytes in the lobules of this group was reduced twofold compared to the control group. [4]

The morphological and morphometric changes in the thymus under chemotherapy indicate that early and rapid involution of the thymus develops, leading to impaired differentiation and maturation of T lymphocytes. Consequently, deficiency of T lymphocytes and their subpopulations in the periphery occurs, resulting in a marked suppression of the immune system of the whole organism. Reduced immunity predisposes the organism to rapid infection and progression of various infectious diseases.

In this experimental group, white outbred rats received paclitaxel at a dose of 0.2 mg/kg and 0.7 ml of pomegranate seed oil intragastrically via a stomach metal probe for 21 days. Morphological changes in the thymus were studied under these conditions.

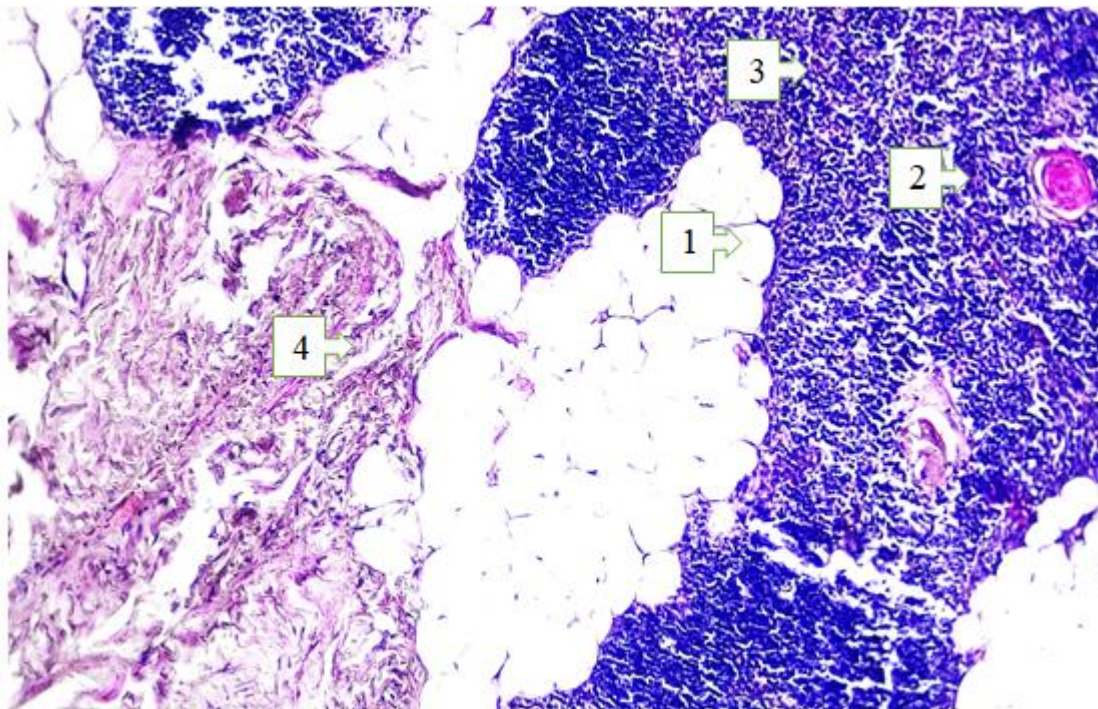
In the third group, it was observed that while thymalin has a strong stimulatory effect on the hematopoietic organs, it simultaneously led to deficiencies in vitamins, minerals, and biological elements in the organism.

It is well known that pomegranate seed oil contains vitamins, unsaturated fatty acids, minerals, and plant extracts. In particular, punicic acid in pomegranate seed oil exerts antioxidant, antiseptic, anti-inflammatory, local, and systemic effects. Under the complex action of pomegranate seed oil,

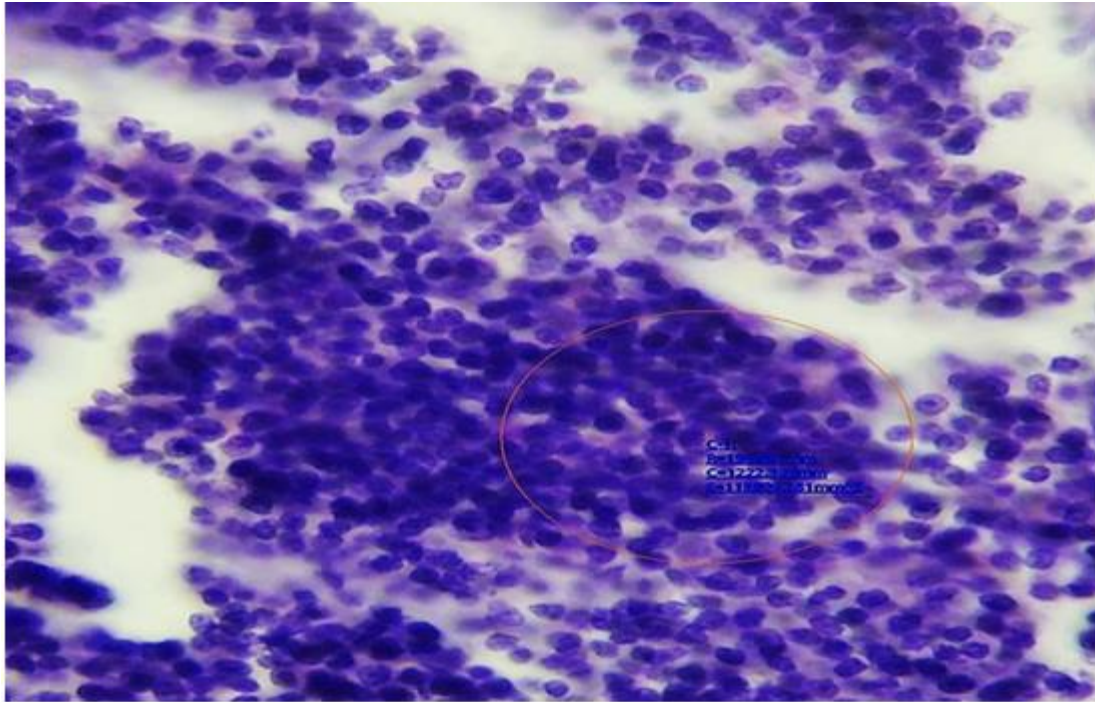
blood supply to cells and tissues improved, vascular regeneration processes were enhanced, circulation throughout organs increased, venous congestion decreased and subsequently disappeared, while tissues became better supplied with nutrients, vitamins, minerals, proteins, and carbohydrates. Therefore, the combined use of a chemotherapeutic drug with pomegranate seed oil was applied to study the correction of morphological changes in the thymic tissue. For this purpose, micropreparations were stained with hematoxylin and eosin and examined.

During the examination of morphological changes in the thymic tissue after combined administration of paclitaxel and pomegranate seed oil, stained with hematoxylin and eosin, the following features were most pronounced. Under the effect of the chemotherapeutic drug, early and rapid involution of the thymus developed, accompanied by apoptosis and necrosis, which disrupted immune system function. Pomegranate seed oil, however, stimulated blood cells and hematopoietic organs, improved systemic circulation, actively participated in phagocytosis of blood toxins, and helped to restore immune system balance. [8]

Autopsy of rats in this group showed that the size and weight of the thymus were comparable to those in the healthy control group. Microscopic analysis of histological preparations at low and high magnifications revealed that the main structural components of thymic lobules—the cortex and medulla—were distinctly stained and differentiated (similar to the control group thymus). [5]



**Figure 3.** Microscopic structure of the thymus in 3-month-old white outbred rats after experimental chemotherapy combined with pomegranate seed oil. Hematoxylin and eosin staining. Magnification 10x20. 1 – Dense accumulation of T lymphocytes in the cortical region; 2 – Enlarged and expanded Hassall's corpuscles; 3 – Enlarged reticuloepithelial cells; 4 – Reduced adipose tissue, replaced by connective tissue



**Figure 4.** Morphometry of thymocytes in the cortical region of the thymus of 3-month-old white outbred rats after experimental chemotherapy combined with pomegranate seed oil. Hematoxylin and eosin staining. Magnification 20x20. 1 – Morphometric measurements of cortical T-lymphocytes (thymocytes)

This indicates that in this experimental group, in addition to peripheral release of T lymphocytes from the cortex into circulation, a considerable number of T lymphocytes were preserved and continued to mature. The medullary region stained light pink, while Hassall's corpuscles were enlarged and increased in number, producing cytokines essential for the maturation of cortical T lymphocytes. In the surrounding tissue, adipose tissue was reduced and replaced by light-pink connective tissue. In the stromal tissue, the interlobular septa appeared similar to those in the healthy group, and the blood–thymus barrier (cytoreticulum) showed almost no morphological differences compared to the control group. [9]

During the morphometric study of thymic tissue in experimental white outbred rats treated with paclitaxel in combination with pomegranate seed oil, the following results were obtained: thymic capsule thickness ranged from  $4.51 \pm 0.6$  nm to  $10.2 \pm 0.5$  nm, with an average of  $7.48 \pm 0.6$  nm; subcapsular zone thickness ranged from  $2.5 \pm 0.3$  nm to  $5.27 \pm 0.7$  nm, averaging  $4.17 \pm 0.2$  nm; trabecular thickness ranged from  $2.09 \pm 0.42$  nm to  $5.01 \pm 0.4$  nm, with a mean of  $3.05 \pm 0.7$  nm. The diameter of interlobular arteries ranged from 15.7 nm to 18.9 nm (average 17.1 nm), veins from 10.6 nm to 25 nm (average 17.8 nm), and postcapillary venules from 5.6 nm to 9.7 nm (average 7.8 nm). [6]

The number of thymocytes in the cortical region varied between 7 and 12 per  $1 \text{ mm}^2$ , with an average of 9 per  $1 \text{ mm}^2$ , while in the medullary region their number ranged from 3.5 to 5.5 per  $1 \text{ mm}^2$ , averaging 4.5 per  $1 \text{ mm}^2$ . When counted in a standard microscopic field, cortical thymocytes numbered from 135 to 198 cells (average  $157 \pm 14$ ), whereas medullary thymocytes ranged from 62 to 115 cells (average  $75 \pm 11$ ). [10]

These results indicate that the number of thymocytes in lobules of this experimental group was higher compared to the second and third groups, though still slightly lower than in the control group. Therefore, the combined use of pomegranate seed oil with chemotherapeutic agents plays an important role in maintaining thymic function and ensuring immune system stability. [11]

## 4. Conclusions

Experimental studies demonstrated that administration of cytostatic drugs such as paclitaxel causes profound morphological and morphometric changes in the thymic tissue. In treated animals, thymus size and weight were reduced, with early signs of organ involution observed. The thymic capsule and trabeculae thickened significantly, blood vessels dilated, and in certain areas hemodynamic disturbances and stasis developed. In addition, the cortical region showed a sharp decline in T-lymphocyte numbers, with increased apoptotic forms and intensified phagocytosis. These changes disrupted the thymus's primary immunogenetic function—the differentiation and maturation of thymocytes. [12]

Morphometric parameters in the paclitaxel group also shifted negatively: capsule and trabecular thickness increased markedly beyond physiological limits, vascular diameters widened, and thymocyte density decreased significantly. These alterations form the morphological basis for immunosuppression and increased susceptibility to infectious and inflammatory processes during chemotherapy. [13]

In the second phase of the experiment, when paclitaxel was administered together with pomegranate seed oil, the severity of thymic pathomorphological changes was notably reduced. Thymic lobules were better preserved, cortical and medullary zones were clearly demarcated, T-lymphocytes were densely arranged, and their proliferative activity was relatively maintained. Hassall's corpuscles were enlarged, showing signs of functional activity. The integrity of the blood–thymus barrier was comparatively well preserved, and hemodynamic disturbances were minimal. [14]

Morphometric analysis confirmed these positive changes: thymic capsule and trabecular thickness approached physiological values, vascular diameters were significantly reduced, and thymocyte density was much higher compared to the paclitaxel-only group. These findings indicate that pomegranate seed oil, owing to its antioxidant, anti-inflammatory, and immunomodulatory properties, serves as an effective protective factor for thymic structure and function. [15]

Thus, pomegranate seed oil alleviates chemotherapy-induced morphological and morphometric changes in the thymus, supports the differentiation and maturation of thymic lymphoid cells, and contributes to restoring the body's immunological stability. These results provide an important scientific basis for the development of strategies to incorporate adjuvant therapeutic agents into clinical oncology practice.

---

## REFERENCES

- [1] Abbas A.K., Lichtman A.H. *Cellular and Molecular Immunology*. Philadelphia: Elsevier, 2021. – 608 p.
- [2] Paltsev M.A., Potapov V.A. *The Immune System and Its Organs*. Moscow: GEOTAR-Media, 2018. – 352 p.
- [3] Vose J.M. Mechanisms of chemotherapy-induced immunosuppression. *Seminars in Oncology*, 2020; 47(2): 110–120.
- [4] Tursunov Kh.Sh., Ikramov A.N. *Morphofunctional Changes of the Thymus under Toxic Influences*. Tashkent: Fan, 2019. – 224 p.
- [5] Oh S.A., Li M.O. T cell biology in thymus and periphery. *Nature Reviews Immunology*, 2021; 21: 198–213.
- [6] Lansky E.P., Newman R.A. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*, 2007; 109(2): 177–206.
- [7] Jurenka J.S. Therapeutic applications of pomegranate (*Punica granatum*): a review. *Alternative Medicine Review*, 2008; 13(2): 128–144.
- [8] Boimirzaev R.A., Khodjanov F.T. Antioxidant properties of pomegranate seed oils in experimental pathology. *Journal of Theoretical and Clinical Medicine*, 2020; 3(2): 45–51.
- [9] Saleem M., Adhami V.M., Mukhtar H. Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *PNAS*, 2005; 102(41): 14813–14818.
- [10] Kadyrova F.Kh., Alieva N.M. *The Influence of Plant Bioantioxidants on the Morphology of Lymphoid Organs in Animals*. Tashkent: Ilm-ziyo, 2022. – 276 p.
- [11] Baliga M.S., Shivashankara A.R., Shetty P., Thilakchand K.R., et al. Antioxidant and anticancer potential of pomegranate. *Food & Function*, 2013; 4(5): 635–642.
- [12] Aripova G.T., Rasulova N.R. Morphology of the thymus under the influence of cytostatics and ways of correction. *Bulletin of BSMI*, 2021; 5(2): 66–72.
- [13] Viuda-Martos M., Fernández-López J., Pérez-Álvarez J.A. Pomegranate and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety*, 2010; 9(6): 635–654.
- [14] Yusupov Sh.M., Temirova N.R. Morphometric changes of the thymus during experimental chemotherapy. *Morphology*, 2023; 164(1): 88–95.
- [15] Zarfeshany A., Asgary S., Javanmard S.H. Potent health effects of pomegranate. *Advanced Biomedical Research*, 2014; 3: 100.