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# Morphological and Histological Changes in the Colon of Rats Following Radiotherapy for Experimental Testicular Cancer and Their Modulation by Tatar Thistle (Cirsium spp.) Tincture

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**Abstract**

**Background.** Radiotherapy is an important component of cancer treatment, but radiation exposure may cause structural damage to normal tissues, including the gastrointestinal tract. Radiation-induced injury of the intestinal mucosa may involve changes in epithelial cells, crypt architecture, and inflammatory cellular responses. The use of natural plant-derived agents for the correction of radiation-associated tissue damage is therefore of interest.

**Objective.** To investigate morphological and morphometric changes in the colon of rats following radiotherapy in an experimental model of testicular cancer and to evaluate the changes associated with Tatar thistle tincture administration.

**Materials and Methods.** Six-month-old white rats were used in the experiment. Experimental testicular cancer was induced using 7,12-dimethylbenzanthracene. The animals were distributed into control and experimental groups, including animals with induced testicular cancer, animals receiving radiotherapy, animals receiving Tatar thistle tincture, and animals receiving combined radiotherapy and Tatar thistle tincture. Colon tissues were examined histologically using hematoxylin and eosin staining. Alcian blue staining was used to assess mucopolysaccharides and goblet cells. Morphometric parameters included crypt depth and the number of goblet, columnar, neutrophil, lymphocyte, and macrophage cells.

**Results.** Radiotherapy was associated with a reduction in crypt depth from 173.51 ± 0.41 μm in the control group to 156.12 ± 0.37 μm. The number of goblet cells decreased from 66.55 ± 0.51 to 61.27 ± 0.54, while columnar cells decreased from 32.62 ± 5.47 to 18.98 ± 3.10. Following combined

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radiotherapy and Tatar thistle tincture administration, crypt depth was 171.24 ± 1.63 μm, the number of goblet cells was 63.75 ± 3.57, and the number of columnar cells was 30.29 ± 2.05. The combined-treatment group also demonstrated increased numbers of lymphocytes, macrophages, and neutrophils in the examined mucosa.

**Conclusion.** Radiotherapy in rats with experimental testicular cancer was associated with morphological alterations of the colonic mucosa, including changes in crypt depth and epithelial cell populations. Administration of Tatar thistle tincture after radiotherapy was associated with partial normalization of the investigated morphometric parameters and changes in the cellular composition of the colonic mucosa.

**Keywords**

*Colon; Radiotherapy; Experimental testicular cancer; Tatar thistle; Tincture; crypt depth; Goblet cells; Columnar cells; Lymphocytes; Macrophages; Neutrophils; morphometry.*

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## 1. Introduction

Malignant neoplasms are multifactorial diseases that develop through the interaction of numerous endogenous and exogenous factors. Despite substantial progress in cancer biology, the mechanisms underlying the transformation of normal cells into malignant cells remain complex (Stricker-Krongrad *et al*., 2026; Siddiqui *et al.*, 2022). Environmental factors and disturbances in the internal regulatory systems of the organism may contribute to the development and progression of malignant neoplasms (Tsiring *et al*., 2023; Liu *et al*., 2024).

Radiotherapy is one of the major therapeutic approaches used in the management of malignant tumors. Its antitumor effect is associated primarily with radiation-induced damage to cellular structures and inhibition of tumor-cell proliferation (Skelton *et al*., 2019; Al-Shammari *et al*., 2025). Ionizing radiation can interfere with mitotic activity, affect microtubular structures and enzyme-dependent processes, and alter the metabolism of nucleic acids, lipids, and amino acids (Delaney and Barton, 2015; Kirsch *et al.*, 2018; Citrin, 2017).

However, radiation exposure may also damage normal tissues. The gastrointestinal tract is particularly susceptible as the high proliferative activity of its epithelial cells (Csiszar *et al*., 2019; Malabadi *et al.*, 2024). Gastrointestinal toxicity is considered an important complication of radiotherapy and may limit treatment intensity (Ghodousi *et al.*, 2023; Albahri *et al*., 2024). Radiation-associated gastrointestinal injury may involve damage to the mucosal epithelium and may persist after the acute phase of treatment (Montay-Gruel *et al*., 2019).

Radiation-induced injury of the intestinal mucosa may manifest as changes in epithelial integrity, crypt architecture, connective-tissue components, and the cellular composition of the lamina propria (Domina, 2023; BAKHRONOV *et al.*, 2021). Experimental morphological and morphometric assessment provides an opportunity to quantify these changes and to evaluate possible corrective interventions (Anwar *et al*., 2020; Situmorang *et al*., 2024).

The search for natural agents capable of reducing radiation-associated tissue injury has attracted increasing interest. Particular attention has been given to plant-derived substances with antioxidant properties (Davronova *et al*., 2024; Bolus, 2017). In the study, Tatar thistle tincture was investigated as a natural corrective agent. The original manuscript describes Tatar thistle as a plant-derived antioxidant and proposes its potential role in reducing tissue damage associated with cancer treatment (Guo *et al*., 2017; Simon and Bouville, 2015).

Previous experimental observations demonstrated morphological alterations in the colon of six-month-old rats with experimental testicular cancer following radiotherapy and administration of Tatar thistle tincture (Khan *et al*., 2019). These observations provided the rationale for a more detailed assessment of the structural characteristics of the colonic mucosa and its cellular components (Sharma *et al*., 2024;

Asma *et al*., 2022).

Although radiation-induced gastrointestinal injury has been investigated experimentally, the study addresses the morphological response of the colon in an experimental cancer model with particular emphasis on crypt depth, epithelial-cell populations, and inflammatory and immune-cell components following radiotherapy and subsequent administration of Tatar thistle tincture.

## 2. Materials and Methods

### 2.1. Experimental animals

White laboratory rats aged six months were used in the experiment. The animals were maintained under standard vivarium conditions at a temperature of 20–22°C, in groups of 5–7 animals, with unrestricted access to food and water.

Before the experiment, the animals underwent a 7-day quarantine period to identify possible somatic and infectious diseases. During the experimental period, the behavioral and physiological condition of the animals was monitored and recorded.

### 2.2. Induction of experimental testicular cancer

Experimental testicular cancer was induced in six-month-old rats using 7,12-dimethylbenzanthracene (DMBA). According to the original manuscript, 0.1 mg of DMBA was administered to the experimental animals. The reported tumor-induction success rate was 70.1%, with testicular cancer developing in 121 animals.

### 2.3. Experimental groups

The original manuscript describes five experimental groups:

| Group | Experimental condition | Number of animals |
|---|---|---|
| I | Control group | 24 |
| II | Experimental testicular cancer | 26 |
| III | Testicular cancer + radiotherapy | 25 |
| IV | Testicular cancer + Tatar thistle tincture | 22 |
| V | Testicular cancer + radiotherapy + Tatar thistle tincture | 24 |

Radiotherapy was administered to Groups III and V for 10 days. Tatar thistle tincture was administered to Groups IV and V at a dose of 0.5 mL.

Important methodological note: The source manuscript reports different total numbers of animals in different sections: 93 animals at the beginning of the Methods section, 172 animals subjected to DMBA administration, 121 animals with induced testicular cancer, and a group distribution totaling 121 animals.

Therefore, the final submitted version should retain one verified sample-size description. This discrepancy should not be resolved by editorial assumption.

### 2.4. Radiotherapy and administration of Tatar thistle tincture

According to the source manuscript, radiotherapy was administered for 10 days. The manuscript describes the radiation exposure as “0.2 g” per day; however, a radiation dose expressed in grams is not sufficiently defined for reproducibility.

Therefore, the final journal version should specify the actual radiation dose and unit, radiation source/device, exposure duration, and other relevant irradiation parameters if these data are available.

Tatar thistle tincture was administered intra gastrically at a dose of 0.5 mL using a metal gastric tube.

### 2.5. Histological examination

Colon specimens obtained from control and experimental animals were subjected to histological examination following staining with hematoxylin and eosin (H&E).

The relative numbers of neutrophils, lymphocytes, plasma cells, fibrocytes, and fibroblasts were assessed per 1,000 cells at a magnification of ×400.

The histological assessment included evaluation of the mucosal epithelium, crypts, lamina propria, submucosa, muscular layer, and serosa.

### 2.6. Histochemical examination

Alcian blue staining was used to evaluate mucopolysaccharides and goblet cells in the colonic mucosa. The histological sections demonstrated the mucosal, submucosal, muscular, and serosal layers, as well as goblet cells.

### 2.7. Morphometric assessment

The following parameters were evaluated:

- crypt depth (μm);
- number of goblet cells;
- number of columnar cells;
- number of neutrophils;
- number of lymphocytes;
- number of macrophages.

The morphometric data were compared between the control group, radiotherapy group, and combined-treatment group.

### 2.8. Statistical analysis

The source manuscript reports statistical significance at $p \leq 0.05$.

However, the original article does not specify the statistical software, statistical tests, data-distribution assessment, or exact method used to calculate the reported values. These methodological details should be added to the final version before journal submission.

## 3. Results

### 3.1. General and organometric characteristics

Following combined radiotherapy and Tatar thistle tincture administration, the body weight of the animals ranged from 239.35 to 245.32 g, with a mean value of 242.86 ± 1.37 g. According to the source manuscript, this value was 2.32% lower than that of the control group and higher than the corresponding values in Groups II, III, and IV by 7.88%, 5.45%, and 2.63%, respectively.

The colon length ranged from 13.29 to 14.92 cm, with a mean value of 14.09 ± 0.10 cm. This value was reported to be 3.69% lower than that of the control group and 14.69%, 4.40%, and 1.78% higher than the corresponding values in Groups II, III, and IV, respectively.

### 3.2. Histological characteristics of the colonic mucosa

Histological examination demonstrated preserved architecture of the colonic mucosa in the control animals. The mucosa was covered by columnar epithelium, and the crypt structures were clearly identified.

Following radiotherapy, structural alterations of the mucosa were observed. The source manuscript describes changes in the epithelial layer, crypts, and connective-tissue components, together with increased numbers of lymphocytes, individual neutrophils, and histiocytic cells in the lamina propria.

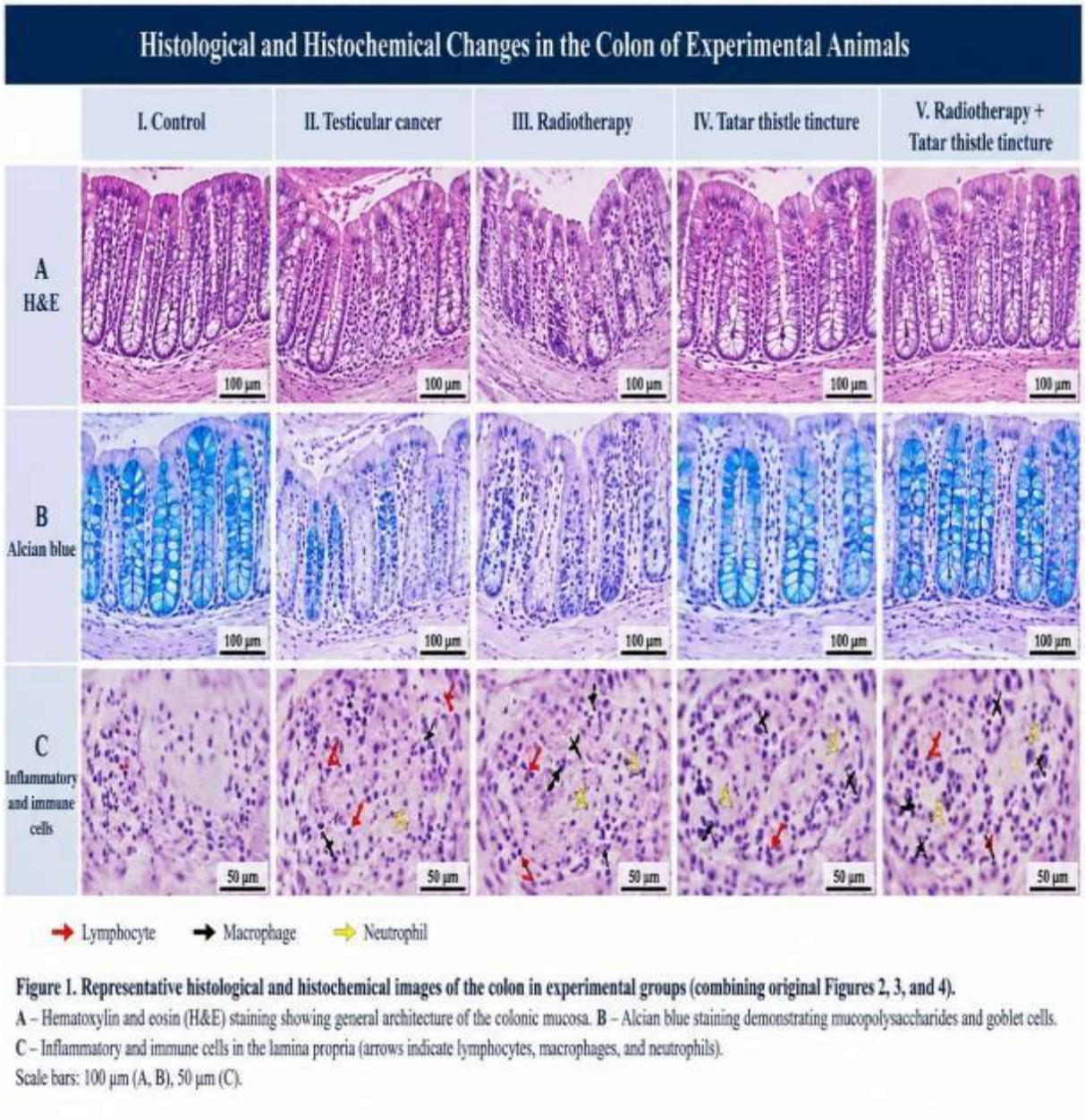



**Figure 1. Representative histological and histochemical changes in the colon of experimental rats.**

A - hematoxylin and eosin (H&E) staining showing the general architecture of the colonic mucosa; B - Alcian blue staining demonstrating mucopolysaccharides and goblet cells; C - inflammatory and immune cells in the lamina propria. Arrows indicate lymphocytes, macrophages, and neutrophils. Scale bars: 100 µm (A, B) and 50 µm (C).

In animals receiving combined radiotherapy and Tatar thistle tincture, the degree of structural damage and inflammatory changes was described as lower than that observed in animals exposed to radiotherapy without correction.

**3.3. Crypt depth and epithelial-cell populations**

The morphometric findings are summarized in Table 1.

A comparative graphical representation of the main morphometric and cellular parameters across the experimental groups is presented in Figure 2.

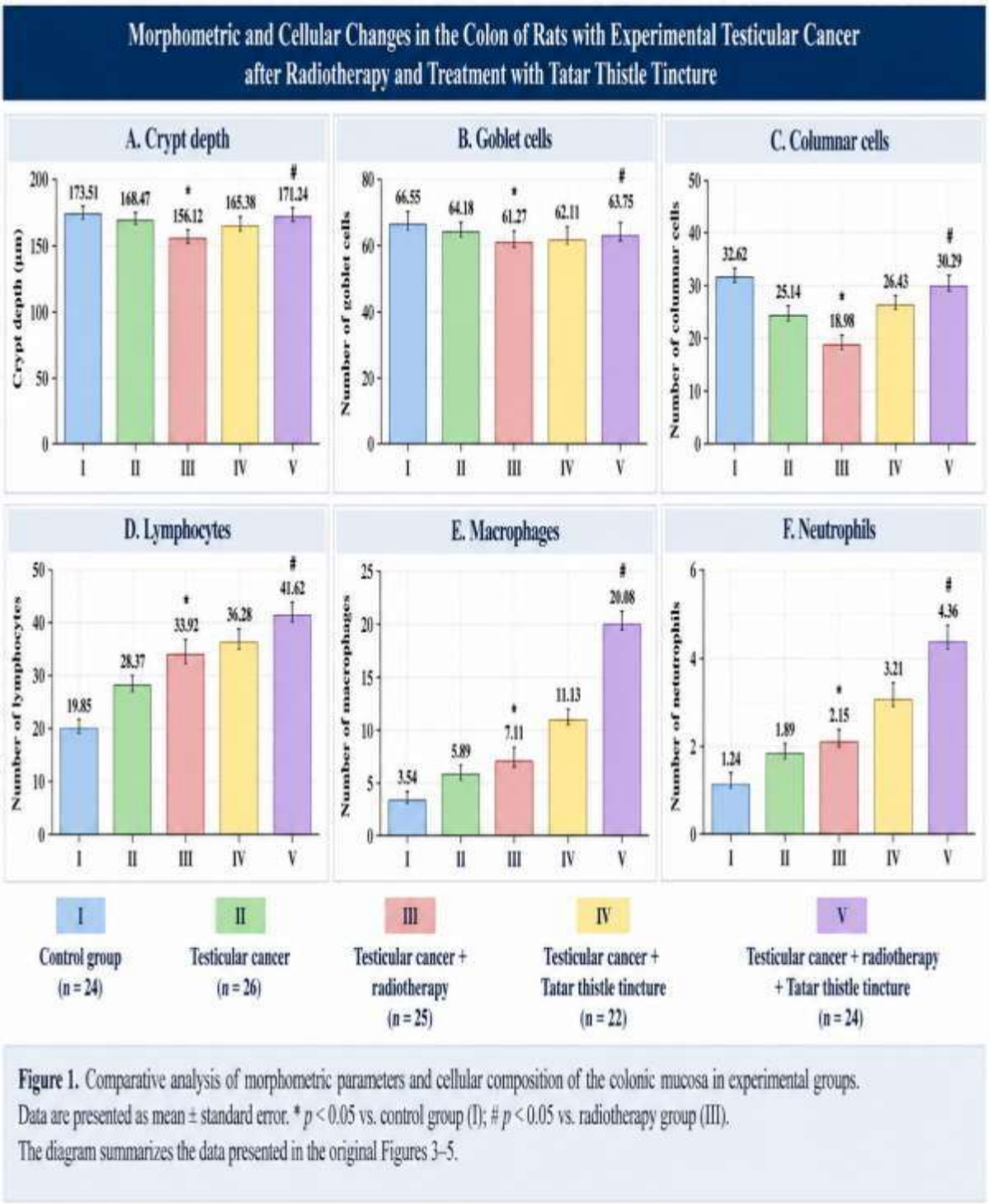



**Figure 1.** Comparative analysis of morphometric parameters and cellular composition of the colonic mucosa in experimental groups.
Data are presented as mean ± standard error. * $p < 0.05$ vs. control group (I); # $p < 0.05$ vs. radiotherapy group (III).
The diagram summarizes the data presented in the original Figures 3–5.

**Figure 2. Comparative analysis of morphometric and cellular parameters of the colonic mucosa in experimental rats.**

Data are presented as mean ± standard error. The figure summarizes the main morphometric and cellular parameters, including crypt depth, goblet cells, columnar cells, lymphocytes, macrophages, and neutrophils, across the experimental groups.

**Table 1. Morphometric characteristics of the colonic mucosa in six-month-old rats**

| Parameter | Control group | Radiotherapy group | Radiotherapy + Tatar thistle tincture |
|---|---|---|---|
| Crypt depth, μm | 173.51 ± 0.41 | 156.12 ± 0.37* | 171.24 ± 1.63* |
| Goblet cells | 66.55 ± 0.51 | 61.27 ± 0.54* | 63.75 ± 3.57* |

| | | | |
|---|---|---|---|
| Columnar cells | 32.62 ± 5.47 | 18.98 ± 3.10* | 30.29 ± 2.05* |
| Neutrophils | 1.24 ± 0.39 | 2.15 ± 0.34* | 4.36 ± 0.70* |
| Lymphocytes | 19.85 ± 0.67 | 33.92 ± 0.69* | 41.62 ± 2.19* |
| Macrophages | 3.54 ± 0.56 | 7.11 ± 0.68* | 20.08 2.15* |

- *$p \leq 0.05$ versus the control group. Source: data reported in the original manuscript.*

The mean crypt depth decreased from 173.51 ± 0.41 μm in the control group to 156.12 ± 0.37 μm following radiotherapy. In the combined-treatment group, the value increased to 171.24 ± 1.63 μm, approaching the control value.

The number of goblet cells decreased from 66.55 ± 0.51 in controls to 61.27 ± 0.54 after radiotherapy. Following combined treatment, the value was 63.75 ± 3.57.

A more pronounced change was observed in columnar cells. Their number decreased from 32.62 ± 5.47 in the control group to 18.98 ± 3.10 after radiotherapy. Following combined treatment, the value increased to 30.29 ± 2.05.

**3.4. Inflammatory and immune-cell changes**

The cellular composition of the colonic mucosa was altered following radiotherapy.

The number of lymphocytes in the combined-treatment group was 41.62 ± 2.19, compared with 19.85 ± 0.67 in the control group and 33.92 ± 0.69 in the radiotherapy group.

The number of macrophages increased from 3.54 ± 0.56 in the control group to 7.11 ± 0.68 following radiotherapy and to 20.08 ± 2.15 in the combined-treatment group.

The number of neutrophils was 1.24 ± 0.39 in the control group, 2.15 ± 0.34 after radiotherapy, and 4.36 ± 0.70 following combined treatment.

The distribution of lymphocytes, macrophages, and neutrophils in Groups III–V is additionally illustrated in Figure 4 of the source manuscript.

## 4. Discussion

The experimental study demonstrated that radiotherapy in rats with experimental testicular cancer was associated with measurable morphological changes in the colonic mucosa. The most pronounced quantitative changes involved crypt depth and epithelial-cell populations.

A reduction in crypt depth from 173.51 ± 0.41 μm in the control group to 156.12 ± 0.37 μm following radiotherapy indicates a structural response of the regenerative compartment of the intestinal mucosa. The crypt epithelium is characterized by active cellular renewal; therefore, changes in crypt architecture may reflect impaired epithelial maintenance following radiation exposure.

The observed reduction in goblet cells and, particularly, columnar cells further demonstrates alteration of the epithelial compartment. The number of columnar cells decreased from 32.62 ± 5.47 in the control group to 18.98 ± 3.10 after radiotherapy. At the same time, the combined-treatment group demonstrated a value of 30.29 ± 2.05, indicating a partial restoration of this parameter.

These findings are consistent with the general concept that radiation may produce structural injury to intestinal tissues. The source manuscript refers to previous experimental observations in which radiation exposure was associated with mucosal atrophy, reduced crypt numbers, and increased fibrosis.

The study also demonstrated changes in the cellular composition of the lamina propria. Following radiotherapy, the numbers of lymphocytes, macrophages, and neutrophils increased compared with controls. Such changes indicate activation of the local cellular response within the intestinal mucosa.

In the combined-treatment group, the number of lymphocytes reached 41.62 ± 2.19, while macrophages reached 20.08 ± 2.15. These findings indicate that administration of Tatar thistle tincture did not simply normalize all cellular parameters toward control values but was associated with a distinct cellular response in the mucosa.

The source manuscript also describes an increase in mast cells, predominantly in degranulated form, and their localization within connective tissue and perivascular regions of the colon.

The possible corrective effect of Tatar thistle may be related to its proposed antioxidant properties.

However, the study did not directly measure oxidative stress markers or antioxidant enzyme activity. Therefore, the antioxidant mechanism should be regarded as a proposed explanation rather than a demonstrated mechanism of action.

Previous studies cited in the original manuscript have investigated different approaches to reducing radiation-induced intestinal injury, including melatonin and metformin, glutamine and hydrolyzed guar gum. The source manuscript reports that these interventions were associated with preservation of intestinal architecture and reduction of radiation-associated injury.

The current findings extend these observations by examining a plant-derived tincture in an experimental cancer model and by combining qualitative histological assessment with quantitative morphometric evaluation. Nevertheless, the study has limitations. The source manuscript does not provide sufficient information regarding the radiation source and physical dose, statistical software and statistical tests, and contains discrepancies in the reported number of experimental animals. These issues should be resolved before final publication.

## 5. Conclusion

Radiotherapy in rats with experimental testicular cancer was associated with structural and morphometric alterations of the colonic mucosa, including a reduction in crypt depth and changes in the populations of goblet and columnar epithelial cells.

Combined administration of radiotherapy and 0.5 mL Tatar thistle tincture was associated with partial restoration of crypt depth and epithelial-cell parameters toward control values. At the same time, changes in the numbers of lymphocytes, neutrophils, and macrophages were observed.

The findings suggest that Tatar thistle tincture may have a potential corrective effect on radiation-associated morphological alterations of the colon; however, further studies with standardized radiation parameters, adequately described statistical methodology, and additional biochemical and molecular markers are required to clarify the mechanisms underlying this effect.

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