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VETERINARY MEDICAL APPLICATIONS OF PHOTOSTIMULATED CHEMOTHERAPY WITH METHOTREXATE IN THE TREATMENT OF MALIGNANT SOLID TUMORS

APLICAŢIILE MEDICALE VETERINARE ALE CHIMIOTERAPIEI FOTOSTIMULATE CU METOTREXAT ÎN TRATAMENTUL TUMORILOR SOLIDE MALIGNE

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ABSTRACT | REZUMAT

Starting from the action of light on the matter, it was determined that there is the possibility of obtaining a selective tumour photostimulation made after the exposure of neoplastic tissues in the field of optical radiation with well-defined parameters.

It has also been assumed that certain antimetabolic cytostatics, after administration, possess the ability to lead to the photochemical generation of free radicals within the nucleus of the tumour cells.

Using these two phenomena, a treatment regimen has been developed that can lead to a significant increase in the therapeutic index of methotrexate.

Key words: photostimulated chemotherapy, methotrexate, carcinoma, cancer

Pornind de la acțiunea luminii asupra materiei, s-a determinat posibilitatea obținerii unei fotostimulări tumorale selective realizate în urma expunerii țesuturilor neoplazice în câmpul unei radiații optice cu parametrii bine determinați.

De asemenea, s-a presupus că anumite citostatice antimetabolice, după administrare, posedă capacitatea de a conduce la generarea pe cale fotochimică a radicalilor liberi, în interiorul nucleilor celulelor tumorale. Prin cuplarea acestor două fenomene s-a realizat o schemă de tratament ce poate conduce la creșterea semnifcativă a indicelui terapeutic al metotrexatului.

Key words: chimioterapie fotostimulată, metotrexat, carcinom, cancer

Due to the fact that the anticancer drugs known to date do not have the total capacity to act in a cancerous process, it is envisaged to develop innovative, personalized treatments aiming at specific targeting of structures for certain types of neoplasm. This experiment aims to put forward an experimental model demonstrating the effect of classical chemotherapy in parallel with classical chemotherapy associated with photostimulated chemotherapy, observing the invasion and tumour metastasis of cells from Walker 256 carcinoma tumours administered to Wistar rats. The drugs used in the treatment schedule exhibit a certain degree of selectivity based on the characteristics of the neoplastic cells, namely: the rate of division and growth, the degree of sensitivity of different types of cells to certain drugs, the rate of drug uptake, the time of tumour doubling (depends on the length of the cell cycle and the growth fraction). This is why we consider the basis for the application of the anti-neoplastic medication to be cell growth and cell cycle. Thus, in the S phase DNA synthesis occurs; Phase M begins with mitosis and ends with cytokinesis; phase G0 represents the non-proliferative phase of the cell cycle. By exposing neoplastic tissues to the field of optical radiation correlated with the administration of certain antimetabolic cytostatics, we want to achieve a treatment regimen that involves a significant increase in the therapeutic index of the used cytostatic, in this case antimetabolite methotrexate that plays a role in inhibiting dihydrofolate reductase required for the formation of tetrahydrofolate, a mandatory cofactor for the synthesis of thymidylate (essential for synthesis and repair of DNA) (2, 3, 4).

MATERIAL AND METHODS

Photostimulation is the cumulative effect on the systemic level obtained from the exposure of tissues in the field of optical radiation of well-defined intensity and wavelength (1). The problem was that of strictly setting the optimal parameters for producing the photostimulation in the radiant power scale and the wavelength of the source used. Using a laser system (Dye-LASER), by scanning the wavelength, the occurrence of the effect was observed in and spectral ranges 400-

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450 nm, 620-680 nm and 760-830 nm, respectively. In all cases, it was found that if the continuous wave power of the generator exceeds 100 mW, the effect disappears (1, 8). Another important aspect is determining the role of radiation coherence in producing photostimulation. Thus, the Laser generator has been replaced by a conventional light source (incoherent) placed at the entrance of a monochromator.

By adjusting the wavelength at previously established intervals, it was found that the effect also occurs in this case, therefore coherence is not a defining property of the irradiation source in the induction of photostimulation (5, 6).

The experimental model consisted in the realization of a series of experimental studies on Wistar rats inoculated with the Walker 256 solid tumour, following the design of a comparative experimental study between the application of classical chemotherapy to Walker 256 solid tumour-inoculated animals and those treated with classical chemotherapy associated with photostimulated chemotherapy (3, 4, 7, 9).

We used 4 male isogenic Wistar rats, obtained and raised in a closed colony at the Biological Institute of the Bucharest Oncological Institute. Mature specimens, weighing 160-180 g at the time of the tumour inoculation, with weight variations of up to 5% within an experimental lot were selected. Animals were inoculated with Walker 256 solid carcinoma, subcutaneously inoculated. Walker carcinoma is a metastatic tumour whose developmental specificity is known and which can provide homogenous tumour material. Subcutaneous grafting was performed with tumour fragments of approximately 3mm^3, freshly harvested from the peripheral region of the transplantation tumour (to avoid transplantation of non-viable tumour cells from the central area commonly exhibiting necrosis). Inoculation was performed under strict sterility conditions. Cell viability was determined by vital colouring with trypan blue. Positive cells were considered unviable as a result of necrosis or destruction during mechanical trituration and were eliminated from the calculation.

The following were used:

1.Walker 256 solid tumour (~ 500,000 cells / 0.5 ml / sample), the cell viability being established by the trypan blue staining method (live cells / intact cells are not stained);

2.Methotrexate solution in 0.9% physiological saline, in a concentration of 10-6 M, dose=0.5 mg /100 g;

3.A special optical irradiation equipment, featuring a mercury vapor lamp with a power of 250 W.

The tumour-bearing animals were treated as follows: two males using classical chemotherapy and the other 2 by classical chemotherapy associated with photostimulated chemotherapy.

RESULTS AND DISCUSSIONS

At the end of the experimental stage, a necropsy was performed in a PSChT treated male that had a good maintenance status, with well-defined tumour, bosselated appearance, expressing massive necrosis with central liquefaction tendency (Fig. 1, 2).

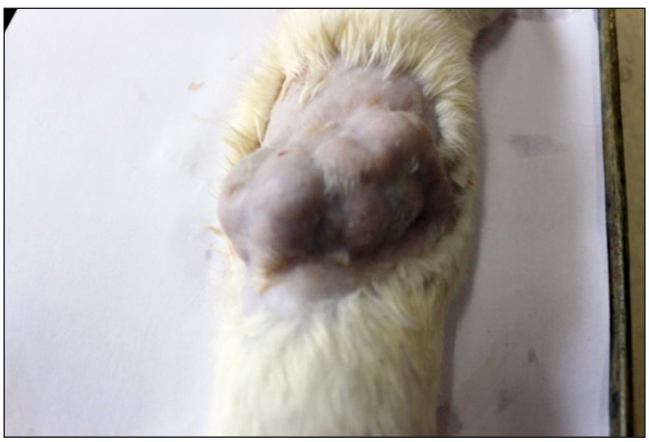

Fig. 1. Delimited tumour

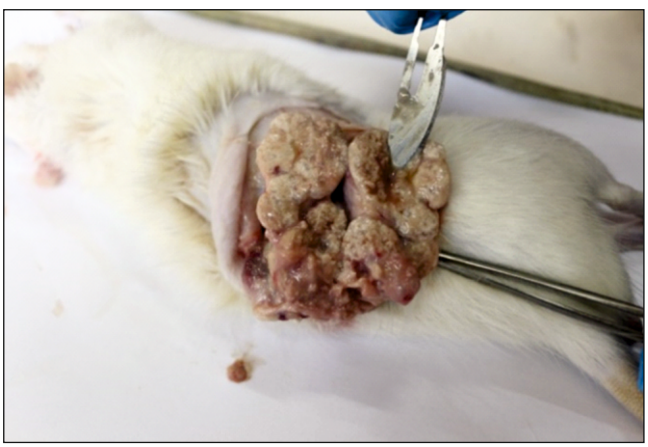

Fig. 2. Caseous necrosis

Severe hepatodistrophy, brittle and discoloured liver was observed. Macroscopically, the spleen and the kidneys did not show significant changes. Also, the inguinal, mandibular and tracheobronchial lymph nodes did not show macroscopic changes. The left and right axillary paraffin lymph nodes were slightly reactive with no color changes.

Samples of the liver, lymph nodes, spleen, and kidney were collected to perform the histopathological examination in an authorized laboratory.

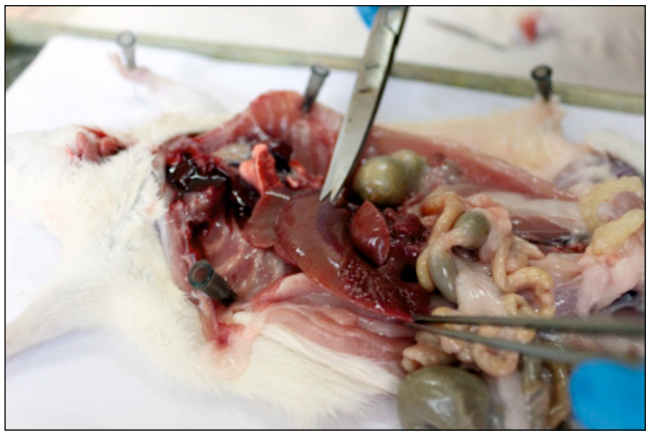

Fig. 3. General appearance of necropsy

Thus, the following were observed:

1. From the histological point of view, the liver did not show significant changes (Fig. 4 and 5)

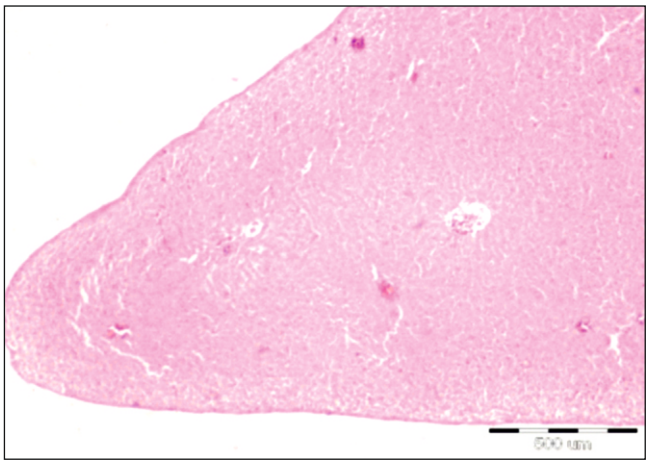

Fig. 4. Liver (10x)

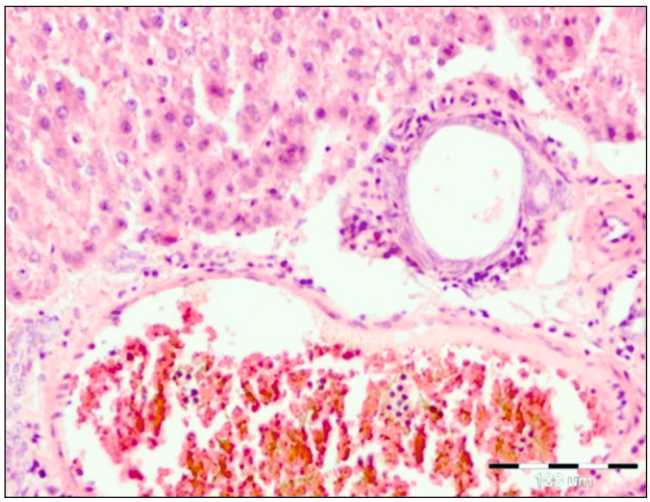

Fig. 5. Liver (20x), biliary port space without changes

2. Metastases at the level of lymph node were found (Fig. 6 and 7)

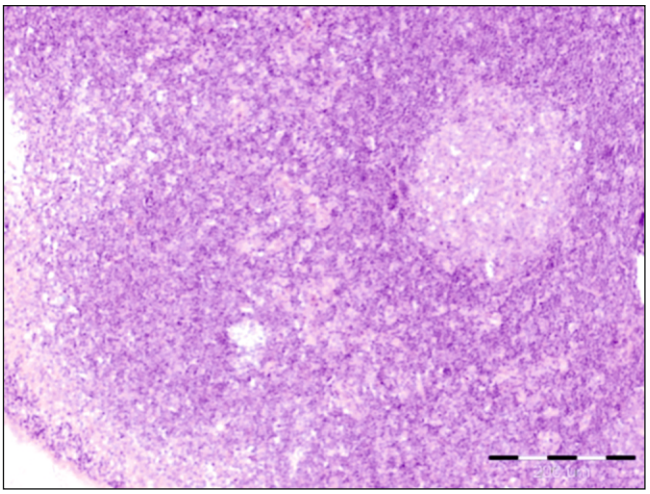

Fig. 6. Lymph node (10x); subcapsular sinus occupied by neoplastic epithelial cells; metastasis

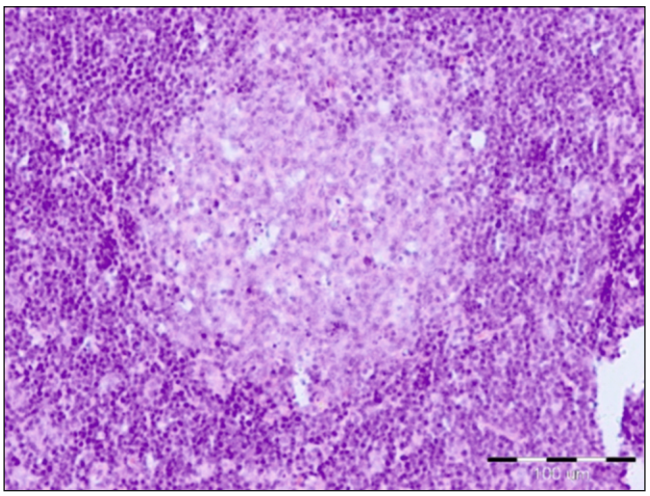

Fig. 7. Lymph node (20x); adenocarcinoma epithelial tumour node metastasis

3. Discrete interfibrilar edema in the myocardium (Fig. 8)

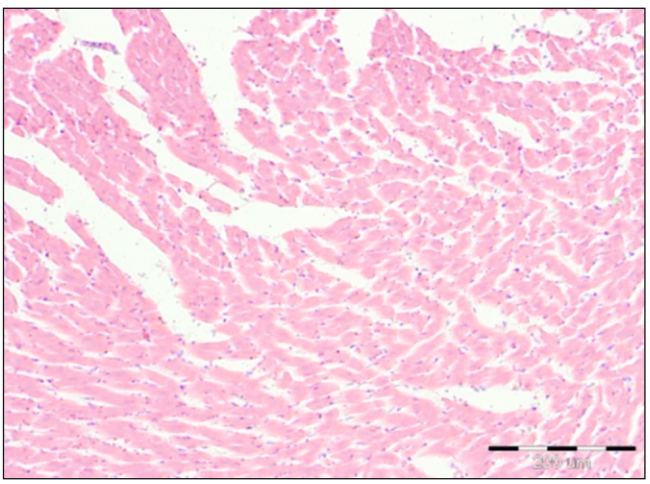

Fig. 8. Myocardium (10x)

4. Pulmonary atelectasis has also been observed, the lung area being reduced in volume and driving in a process of retraction in the neighboring areas (Fig. 9 and 10).

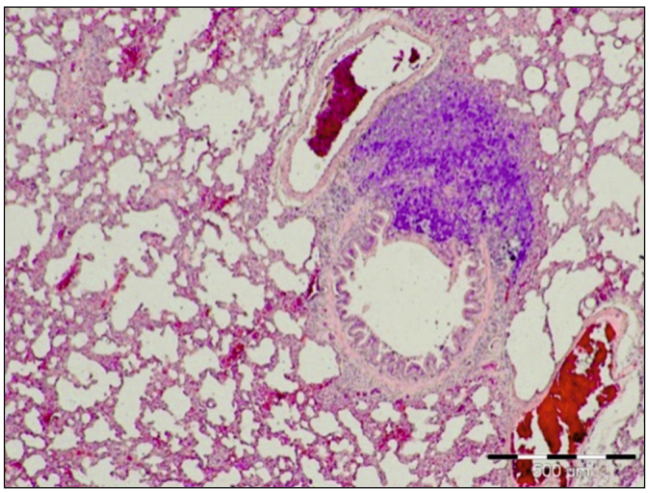

Fig. 9. Lung (4x); multifocal, bulous emphysema, pulmonary atelectasis

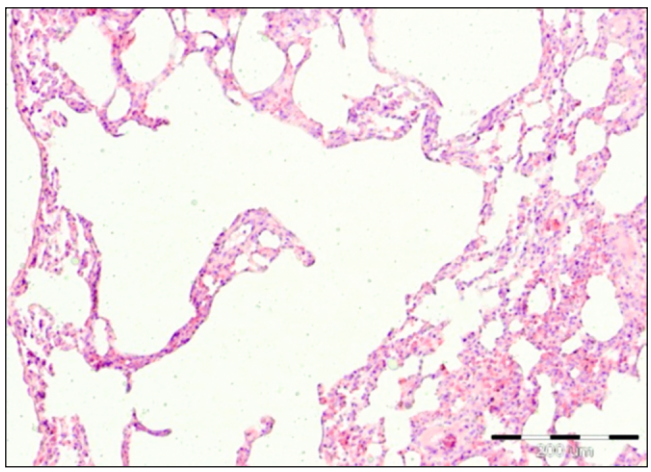

Fig. 10. Lung (10x); bulous emphysema

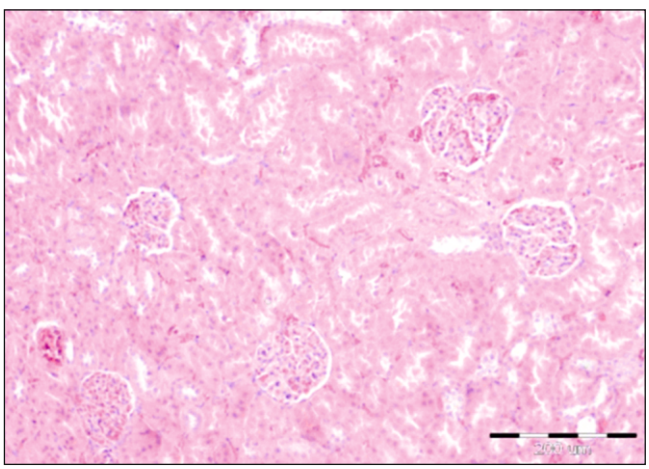

Fig. 11. Kidneys (10x)

5. The kidneys showed moderate diffuse congestion, with an increased number of glomerular mesangial cells (Fig. 11 and 12).

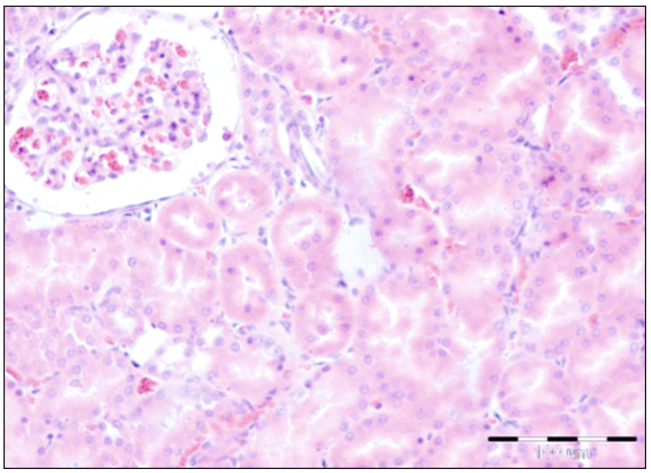

Fig. 12. Kidneys (20x)

6. It has been observed a congested spleen, splenic follicular activation, the ratio of the white pulp and red pulp of 2:1 (Fig. 13).

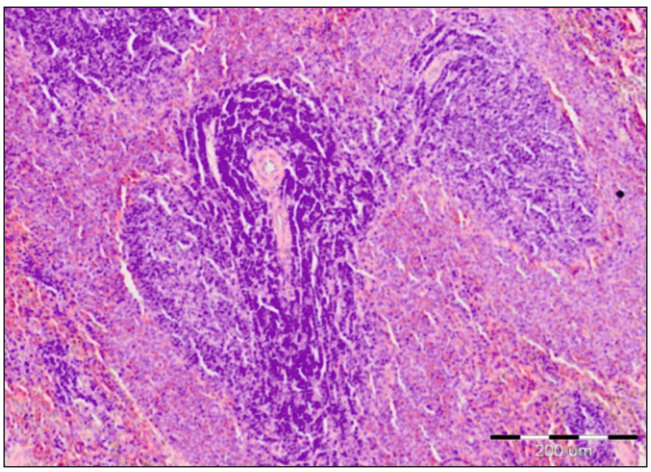

Fig. 13. Spleen (10x)

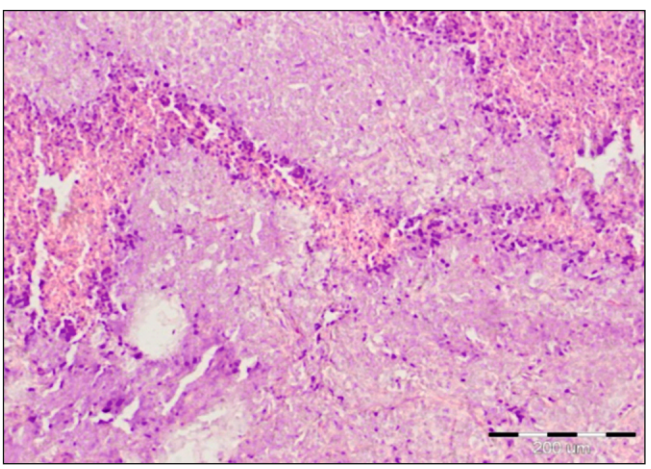

Fig. 14. Neoplastic process with poorly individualized cells

The tumor was characterized as a neoplastic process with a solid pattern consisting of poorly individualized cells (Fig. 14), delimited by a lymphocyte infiltrated pseudocapsula (Fig. 15) consisting of neoplastic cells with malignant morphology presenting anisocariosis and anisocytosis (Fig. 16).

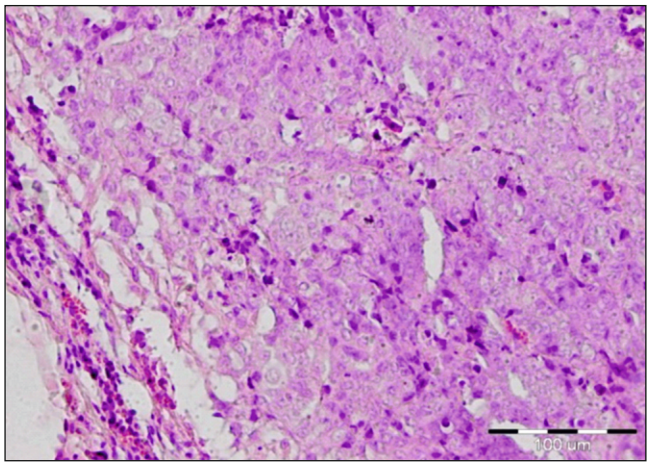

Fig. 15. Neoplastic process delimited by a lymphocyte infiltrated pseudocapsula

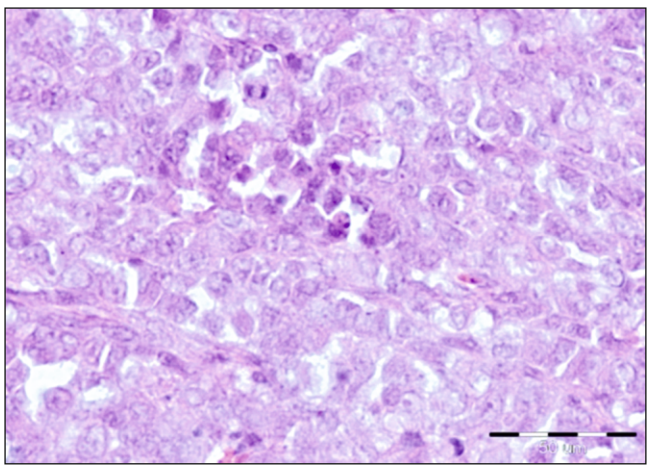

Fig. 16. Neoplastic process with anisocariosis and anisocytosis

CONCLUSIONS

As a result of the above findings, there is a good correlation between the results obtained in the application of the chemotherapy-photobiostimulatory treatment and the theoretical premises of the physico-chemical action of the optical radiation on the methotrexate (biostimulative and photodynamic).

On the other hand, laboratory tests performed on tissues taken from the sacrificed animal that underwent PsChT therapy indicate the appearance of major microscopic changes in the tumour and lymph node. Following the described experiment, the optimal technical parameters (cytotoxic dose irradiation times and chronotherapy elements) that were used in experiments were confirmed. The viability of the method was observed due to the replicability of the results, the survival rate over 90 days being 90% and the total healing of 45%. The experimental model sought to demonstrate the viability of the hypotheses regarding the possibility of increasing the therapeutic index using the Photostimulated Chemotherapy Method.

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