EFFECTS OF PHYTOPREPARATION BE ON THE LYMPHOCYTE QUANTITY IN THE IRRADIATED ANIMALS

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In this research were studied the effects of phytopreparation Be (triterpenoid received from the bark of birch Betula pendula Roth) on the lymphocyte quantity in bone marrow, spleen, lymph nodes of small intestine and thymus after acute sublethal gamma radiation. Analysis of the test results showed that phytopreparation Be increases the number of lymphocytes in spleen and lymph nodes of small intestine in the irradiated organism, normalizes their quantity in bone marrow and does not affect thymus.

Ionizing radiation causes structural changes in immune components. In addition, it provokes acute functional disruptions, that lead to inadequate immune responses [9]. This results in a secondary immune deficiency, accompanied by autoallergic reactions, opportunistic infections, oncological pathology and faster aging [1,2]. A significant feature of the immune system – its relatively low ability to repair radiation-induced damages – is an additional factor, contributing to appearance of the above mentioned complications. An important role in immunogenesis play lymphoid organs of the immune system and migration in central and peripheral parts of the immune system. Lymphoid tissue is a central organ of immunity, marked by uncommonly high level of sensitivity to different external influences, which reduce the immune function. That means a suppression of the T-system, along with humoral and nonspecific phagocytic components of the immunity [14,16,17]. Radiation has a damaging effect on the lymphatic system, and causes immune dysfunctions in different components of the immunity. Due to that fact immune deficiencies may occur; and thus the existing disruptions in the lymphatic system require to be corrected. One of the current challenges for specialists in radiobiology and clinical medicine, is a search for new methods of effective anti-radiation protection. An important place among those have medications for correction of immune and metabolic disruptions in the irradiated body [7,15]. Birch is a valuable source of biologically active substances, some of which have long being used in the medical practice. Birch bark, buds, young leaves, juice, fungus are tar can be used for the treatment purposes. Birch bark contains a special coloring agent triterpene betulinic alcohol, glycosides and betulosides, gaultherin, phytosterol, saponins, tannin, tanning agents, alkaloids and essential oils. On basis of betulin, contained in birch bark, scientists from Novosibirsk have developed a preparation with a preliminary name «Birch juice» for the treatment of HIV-positive persons. According to N.I. Maznev [11] and G.P. Yakovlev [19], it has a diuretic, cholagogic, diaphoretic, disinfectant and antiseptic effect. Bark is also used to treat dropsy, gout and lung diseases. Birch bark is used as a wound healing and disinfectant remedy for the treatment of abscess, furunculosis, rash and dermatophytes. Spirit extract on basis of bark and young sprouts has a high antioxidant activity [5].

The objective of this research was to study, how phytopreparation triterpenoid with a labor code Be, received from the bark of silver birch (Betula pendula Roth), influences on the lymphatic system after acute total gamma radiation.

3 sets of experiments were conducted using 45 white mature outbred rats (weighted 180-200 g). The 1st group consisted of intact animals (n=15), the 2nd group (control group) included irradiated animals (n=15), the 3rd group (experimental) consisted of animals, who received radiation + phytopreparation Be (n=15). All test animals were housed in similar vivarium conditions. The animals from the 2nd and the 3rd groups were irradiated at a dose of 6 Gy, by gamma rays ^{60}Co.

The irradiation was generated with a radiotherapy machine «Agat-RM». Tests in the 2nd group were carried out 30 days after the irradiation. In two weeks after the irradiation, animals from the 3rd group were given phytopreparation Be (25 mg/kg of body mass) per os during 14 days. In order to determine the quantity of lymphocytes in spleen, thymus, bone marrow and lymph nodes of small intestines, homogeneous suspensions were prepared, obtained from irradiated, not radiated and with phytopreparation treated animals. Cells in bone marrow were measured, using a method of Gorizontov P.D. and co-authors [6]. The number of thymocytes was determined according to Belousova O.I. and Fedotova M.I. [3]. The quantity of lymphoid cells in spleen and lymph nodes of small intestines was measured using a method of Goldberg E.D. with co-authors [4] and Zhetpisbaev B.A. [8]. The indices were determined in all 3 groups; the figures were statistically processed by the Montsevichute-Eringene E.V. statistical method [12], using Student's t-tests. The difference was regarded as reliable when $P \leq 0,05$.

Results of the research on the phytopreparation Be's effects on the lymphocyte quantity in immunocompetent organs and tissues in the irradiated animals are shown in Table 1.

Table 1. Effect of phytopreparation Be on lymphoid organs of immune system in the irradiated organism.

Index	1 Intact animals (n=15)	2 Irradiated animals (n=15)	3 Irradiated animals+ phytopreparation Be (n=15)
Bone marrow	0,16+0,041	$0,31+0,053^{0}$	0,20+0,023*
Thymus	9,2+0,28	$3,9+0,6^{0}$	$7,9+0,34^{*0}$
Spleen	2,5+0,39	$3,6+0,24^{0}$	$8,5+0,16^{*0}$
Lymph nodes of small intestines	0,72+0,031	$0,58+0,025^{0}$	$1,1+0,22^{*0}$
Commentary: ^{0} – true for the 1st group (P<0,05), * - true for the 2nd group (P<0,05), lymphocytes 10^{6}			

Table 1 shows that under the influence of gamma-radiation the quantity of lymphocytes increases in bone marrow by 93,7% and in spleen by 44%, the number of cells falls in thymus by 57,6% and in lymph nodes of small intestines by 19,5% (P<0,05). Total gamma-radiation of white rats at a dose of 6 Gy let assume that radiosensitivity of the lymphatic system grows in the following order: thymus – lymph nodes - spleen – bone marrow. According to some authors, the whole lymphatic system shows a significant destruction of lymphocytes [18,20]. The immune system responds to developing pathological process with changes in migration ability of central and peripheral lymphoid system, which can be regarded as a general physiological reaction of organism to radiation [13].

Under the influence of phytopreparation Be, the number of lymphocytes in bone marrow decreases reliably till the level of the intact group; in thymus, the amount of cells grows reliably by 102,6% (P<0,05), compared with control group, but this figure does not reach the level of the group with intact animals. In spleen and lymph nodes of small intestines the lymphocyte quantity increases reliably by 136,1% and 89,6% correspondingly (P<0,05) in comparison with control group, and these figures are higher than in the group with intact animals.

To sum up, phytopreparation Be, when given to an irradiated organism, increases the

number of lymphocytes in spleen and lymph nodes of small intestines, normalizes their quantity in bone marrow and has no impact on thymus. The growing number of lymphocytes in the examined organs and tissues appears to be a mobilization of organism's adaptive reactions, when animals find themselves under the influence of pathological impacts (ionizing radiation).

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