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THE EFFECT OF THE *EMINIUM REGELII* EXTRACT ON CELLULAR IMMUNITY

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Abstract. The article presents the results of a study on the effects of the wild medicinal plant *Eminium regelii* Vved. extract to the body of intact and subjected to emotional stress animals on the background of a long-term low dose of gamma-radiation. The reaction of the animal organism to the effects of radiation, emotional stress and the introduction of the *E. regelii* extract were evaluated by the total content of leukocytes, absolute and relative content of lymphocytes (including CD3+, CD4+, CD8+), immunoregulatory index (IRI) and leukocyte migration inhibition reaction (LMIR). The experiments were performed on adult mongrel white rats of both sexes. At the early stage of the general adaptation syndrome (GAS) the injection of *E. regelii* extract caused a decrease in the total number of lymphocytes, an increase in the number of CD4+ lymphocytes and leukocytes lymphokinproduction abilities. Late stage of GAS was indicated by increased CD3+ and CD4+ lymphocytes, and increased values of the immunoregulatory index.

Keywords: emotional stress, gamma-radiation, general adaptation syndrome, immunoregulatory index, leukocytes, leukocyte migration inhibition reaction, lymphocytes

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INTRODUCTION

The human body is constantly exposed to ionizing radiation from both natural and artificial sources. There is a variety of published data on the effects of radioactive radiation, some of which are contradictory. For example, some researchers believed that low doses of radiation have a positive stimulating effect and lead to a radiation hormesis (Buldakov & Kalistratova 2005; Luan et al. 2006; Monfared et al. 2006; Luckey 2007; NAIR 2009; Nomura et al. 2013; Gapeyev et al. 2015; Hekim et al. 2015; Vivek Kumar et al. 2015; Cho et al. 2016)

Other studies denied any stimulating effects of any radiation dose and are convinced in the pathological reaction of the organism to radiation effects (El-Halim et al. 2015; Ivanov et al. 2015; Miñana et al. 2015; Oujifard et al. 2015). However, it is commonly accepted that radiation doses cause different functional changes in the cells, and subsequently, at the organismal level through the changing of the immunological reactivity of animals.

The phytotherapy of negative effects caused by ionizing radiation is also interesting. The use of herbal remedies is an alternative to the chemical radioprotectors that reduce the harmful effects of radiation therapy, at the same time causing series of adverse effects on humans (Islamian & Mehrali 2015) In the Central Asian medicinal plant *Eminium regellii* Vved. the flavo-noids luteolin and quercetin were discovered (Silybayeva et al. 2014) They are known to inhibit in vitro the growth of cancer HCT-15 cells (Zharykbasova et al. 2015). Therefore, it is promising to study the effect of the extract of this species and the long-term effect of the emotional stress on the cellular immunity of irradiated animals.

MATERIALS AND METHODS

The experiments were carried out on 170 white, not purebred adult rats weighting an average of 180 ± 20 g, which were divided into 5 groups. Group 1 – intact animals (n = 15), 2nd – irradiated animals of the long-term period (n = 20), 3rd – the intact animals, affected by emotional stress (n = 45), 4th – irradiated animals of the long-term period, affected by emotional stress (n = 45), 5th – irradiated animals of the long term, affected by emotional stress and treated with *E. regelii* extract (n = 45). The fifth group received a course of *E. regelii* extract at 2,5 mg kg^{-1} intragastrically with the help of gavage once a day (in the morning on an empty stomach) during a period of fourteen days. The dose of irradiation of animals from the 2nd, 4th and 5th groups with gamma-rays was ^{60}Co 0.2 Gr and was carried out on the Russian radiotherapy apparatus *Agat-RM* by gamma rays ^{60}Co. Assessment of the long-term immune status was performed after three months of the radiative effect of a dose of 0.2 Gr.

We used the rat tail hanging for an hour as emotional stress trigger. Blood sampling was carried out after one, two and three days after provoked emotional

stress. Blood samples were taken into tubes with heparin (25 U ml^{-1}) to measure the immune status. Isolation of lymphocytes from venous blood was performed by the conventional method (Garib et al. 1995) in a density gradient ficoll-verografin (1,077). The reaction inhibition of leukocyte migration (RILM) to phytohemaglutinin (PHA) was determined by the method of Artemova (1973). The emotional stress was provoked by the method by Zhetpisbayev et al. (1999). Immunological parameters were determined after one, two and three days after post-stress reaction.

Digital data were processed by standard methods of variation statistics (Montsevichyute-Eringene 1961).

The condition of cell immunity was assessed by the flow cytometry and differentiating functions of mytohen productional inhibition reaction by the number of total CD3+, CD4+ and CD8+ lymphocytes with appropriate monoclonal antibodies. The immunoregulatory index (IRI) was calculated. The principle of the method is to attach the human erythrocytes sensitized with monoclonal antibodies LT to the lymphocyte surface.

RESULTS

Within three months after the effect of low dose gamma radiation, normalization of the total number of white blood cells was observed, as well as a significant increase in the number of lymphocytes (**Table 1**). Statistically reduced, in comparison to the control group, remained the number of both relative and absolute numbers of CD3+ lymphocytes.

Table 1. T-immune system in the late period after low dose gamma irradiation.

Indicators, in 1 µl		1st group of animals (Intact)	2nd group of animals (Irradiated)
Leukocyte, absolute number		6520±150	6055±122
Lymphocytes	absolute number	2800±113	3792±115*
	portion of the total number of leukocytes, %	40±3,6	57±2,2*
CD3+ T-Lymphocytes	absolute number	1457±84	875±40.9*
	portion of the total number of lymphocytes, %	32±2,2	22±1,7*
CD4+ T-helpers	absolute number	698±45,9	477±25,9*
	portion of the total number of lymphocytes, %	21,2±1,9	18±1,2
CD8+ T-suppressors	absolute number	488±22,0	593±19,9*
	portion of the total number of lymphocytes, %	10,8±0,6	11±2,9
IRI (CD4+/CD8+)		1,96±0,16	1,6±0,24
LMIR (index)		0,8±0,06	0,72±0,01
Note: * - the differences from baseline were significant (P <0.05)			

Thus, the subpopulations of T-lymphocytes were different: absolute number of T-lymphocyte with helper activity was reduced to 32%, but the absolute number of T-lymphocyte with suppressive activity, in contrast, increased to 19% (P <0.05). This change caused a reduction of IRI to the control level. The lymphokinproductional ability of white blood cells corresponded to the control level.

At the same time, the analysis shows that, under the low doses of long-term gamma radiation, on the background of recorded lymphocytosis, the reduction of a subpopulation of T-lymphocytes with helper and T-lymphocytes increased with the increase of the suppressor activity.

T-system on the period of the stress effect reacted as follows **(Table 2)**: after 1st day of the stress, the leukocytes in the peripheral blood of irradiated animals were significantly reduced (1.45 times), the number of lymphocytes was reduced 2.26 times, the absolute number of CD3+ - 1.87 times, CD4+ - 1.6 times, CD8+ - 3.1 times, in comparison to the irradiated animals not treated by emotional stress (2nd group of animals). IRI and LMIR to PHA did not change much, the value of the latter was lower than the intact level.

Table 2. The indicators of the T-system after the long-term effects of a low dose of gamma radiation and of the emotional stress.

Indicators, in 1 µl		1st group of animals (Intact)	2nd group of animals (Irradiated)	Indicators after stress	
				after 1 day	after 3 days
Leukocyte, absolute number		6520±15	6055±122	4180±102*^{0}	10636±250*^{0}
Lympho-cytes	absolute number	2800±113	3792±115*	1672±37,6*^{0}	4999±120*^{0}
	portion in the total number of leukocytes, %	40±3,6	57±2,2*	40±2,5^0	47±2,3^0
CD3+ T-Lympho-cytes	absolute number	1457±84	875±40.9*	468±59*^{0}	1291±112^0
	portion in the total number of lymphocytes, %	32±2,2	22±1,7*	29±1,7^0	26,3±1,5*
CD4+ T-helpers	absolute number	698±45,9	477±25,9*	297±10,7*^{0}	712±65,4^0
	portion in the total number of lymphocytes, %	21,2±1,9	18±1,2	16,6±1,2*	14,3±1,4*^{0}
CD8+ T-suppres-sors	absolute number	488±22	593±19,9*	191,6±12,3*^{0}	578±42,1
	portion in the total number of lymphocytes, %	10,8±0,6	11±2,9	12,3±2,7	12,1±2,2
IRI (CD4+/CD8+)		1,96±0,16	1,6±0,24	1,6±0,34	1,2±0,31*
LMIR (index)		0,8±0,06	0,72±0,01*	0,74±0,015*	0,73±0,003*

Note: * - difference from the intact level is right (to 1 group) (P <0.05);
^{o} - the differences from baseline are right (to 2 group) (P <0.05).

After two days, the number of lymphocytes considerably increased (1.53 times). The CD4 + and CD3+ increased 1.43 and 2.0 times, respectively. The number of CD8+ and IRI did not undergo substantial changes, while LMIR to PHA tended to increase in comparison to the initial level.

After three days of stress, the total number of lymphocytes and CD3+ cells in the peripheral blood remained at a high level, exceeding the initial reference levels. The absolute number of CD4+ cells was 1.49 times higher than the control level and corresponded to the intact level; the number of CD8+ cells remained normal and this reliably caused a 1.63 times reduction in the immunoregulatory index. The indicator of LMIR to PHA was significantly higher than the respective level of the intact group.

These data allowed to conclude that the effect of a low dose of gamma radiation applied in a long-term period, in the early stages of the adaptation syndrome was marked by lymphopenia and reduced subpopulations of CD3+, CD4+ and CD8+ lymphocytes and increased lymphokinproductional ability of white blood cells. In the later stage of stress reaction, it was marked by lymphocytosis, by the rise of the absolute number of CD3+ and CD4+ lymphocytes, normalization of CD8+ cells and lymphokinproductional ability of leukocytes, and by the reduction of the immunoregulatory index.

The effect of the *E. regelii* extract and emotional stress on the irradiated body in a small dose of gamma radiation applied in a long-term period, was that the number of white blood cells in the early stage of the general adaptation syndrome (GAS) was considerably lower than in the intact group (**Table 3**). In the later stage of the GAS, the number of white blood cells was 1.28 times higher than this in the control group. The number of lymphocytes at an early stage of GAS exceeded the original level and remained at a high level in the later stage of the GAS.

In the first day after the stress effect, there were no notable changes in a part of CD3+, CD4+ and CD8+ lymphocytes in comparison to the baseline characteristics. In the case of the control group, the absolute and relative numbers of CD4+ -and CD8 + lymphocytes declined.

Two days after the stress effect, the absolute number of CD3+ was markedly higher than in the original and control group levels. The number of CD4+ and CD8+ was noticeably lower than the control group indexes.

Three days after the stress effect, the absolute and relative numbers of CD3+ lymphocytes were higher than in the control group, the absolute number of CD4+ lymphocytes was higher than the control group index, the number of CD8+ corresponds to the original and control group indexes.

Under the influence of the *E. regelii* extract and emotional stress on the irradiated body at a dose of 0.2 Gr, the immunoregulatory index throughout the monitoring was higher than the control group values. At the backdrop of the *E. regelii* extract in the early stages of the GAS the index of the leukocyte migration was lower than the control group values but three days after the stress effect this indicator tended to increase.

Table 3. The effect of *E. regelii* extract on the cellular part of the immune system in the late period after the combined effect of a low dose gamma radiation and emotional stress.

Indicators	Groups of rats	Intact animals		Indicators after emotional stress					
				after 1st day		after 2nd day		after 3rd day	
		The absolute number	The relative number, %	The absolute number	The relative number, %	The absolute number	The relative number, %	The absolute number	The relative number, %
Leukocytes	1	6,52±0,15		9,26±0,82*		8,32±0,75*		5,11±0,35*	
	2	6,05±0,12	-	6,02±0,46+	-	6,24±0,51+	-	6,13±0,52	-
	3	-		6,98±0,57+		6,55±0,43+		6,57±0,45+	
Lymphocytes	1	2,76±0,12	39,02±3,23	4,55±0,41*	45,65±2,88	3,54±0,28*	44,33±3,65	2,28±0,20	37,61±3,02
	2	3,80±0,11	57±2,2	4,43±0,42*	44,32±4,66	3,77±0,27*	41,11±3,54	3,15±0,20+	42,23±3,65
	3	-	-	3,87±0,41*	43,65±3,27	3,23±0,22	40,23±3,64	3,28±0,22+	41,28±3,47
CD3+	1	1,46±0,10	31,82±2,41	1,86±0,17*	36,44±3,11	1,12±0,09*	21,57±2,03*	0,87±0,09**	22,66±2,05*
	2	8,75±0,40	22,00±1,70	1,43±0,11	25,33±2,11*	1,34±0,12	28,56±2,23+	1,10±0,11*	27,35±2,35
	3	-	-	1,53±0,12	29,65±2,07	1,64±0,08*+	30,37±2,17+	1,53±0,14++#	29,62±2,17+
CD4+	1	0,70±0,04	20,93±1,41	1,02±0,11*	22,66±2,21	0,98±0,08*	19,31±1,77	0,60±0,07	18,77±1,57
	2	0,47±0,02	18,00±1,20	0,57±0,06++	15,82±1,63*+	0,61±0,05+	17,67±1,54	0,56±0,05*	16,77±0,91
	3	-	-	0,73±0,05+#	19,05±1,53	0,74±0,04#+	21,05±1,87	0,73±0,06#	19,58±1,37
CD8+	1	0,49±0,02	11,25±0,98	0,95±0,08***	14,25±1,35	0,81±0,07**	10,27±0,97	0,47±0,03	9,88±0,91
	2	0,59±0,01	11,00±2,90	0,39±0,04+	9,54±0,92+	0,44±0,04+	11,08±0,84	0,45±003	10,22±0,91
	3	-	-	0,44±0,03+	10,33±0,83+	0,45±0,03+	13,14±1,02+	0,46±0,03	10,37±0,85
IRI	1	1,44±0,11		1,07±0,12*		1,19±0,11		1,20±0,11	
	2	1,60±0,24	-	1,43±0,12+	-	1,38±0,11	-	25±0,10	-
	3	-		1,63±0,11+		1,64±0,08+		1,55±0,12+#	
LMIT (index)	1	0,79±0,04		0,67±0,07		0,70±0,06		0,85±0,08	
	2	0,72±0,01	-	0,89±0,04**+	-	0,85±0,06+	-	0,90±0,08	-
	3	-		0,75±0,05#		0,70±0,04#		0,81±0,06	

Note:
Groups of rats: 1 - intact animals are affected by emotional stress; 2 - irradiated animals are affected by emotional stress; 3 - irradiated animals are affected by emotional stress and received *Eminium regelii*;* - Reliably to the original (to the intact animals) (P <0.05); ** - Reliably to the original (to the intact animals) (P <0.01); *** - Reliably to the original (to ithe ntact animals) (P <0.001); + - Reliably to the first group (P <0.05); ++ - Reliably to the first group (P <0.01); # - Reliably to the second group (P <0.05).

In conclusion, the *E. regelii* extract in a dose of 2.5 mg kg^{-1} of body weight in the early stage of the GAS caused a decrease in the total number of lymphocytes, but lead to increase of the number of CD4+ lymphocytes and leukocytes lymphokinproductional ability. Late stage of GAS was demonstrated by increased CD3+, CD4+ lymphocytes and increased values of the immunoregulatory index.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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