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Radio modification of Hemopathology Affected by the Application of Biologically-Active Pectin Containing Composition.

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ABSTRACT

Taking into account biological nature of acute radical damage mass medulloblasts mortality conditioned of radiation induced toxic radicals attack, antitoxic and antiradical action of pectin and chitin containing medication we undertook current studies aimed at correction hematopoiesis of irradiated animals on the back of api-phyto protector based on pectin and chitin containing feed supplement application. Experiments were carried out on 90 white outbred rats with live weight of 180-210 g. divided into 4 groups: intact (1), irradiated(2), api-phytoextract + irradiation(3), irradiation + apy-phyto extract(4). The animals were exposed to single gamma irradiation on the hamma ray unit "Puma" at a dose of 8,0Gy. During 10 days before irradiation (the 3 group) and during 10 days after irradiation (the 4 group) animals were injected 10% api-phyto extract with water or feed at dose 5-7,5 ml/kg. The animals were dead with etherization and in the dynamic researched total number myelocytesin bone marrow of femoral bone. In cell smear of bone marrow, we counted percentage of certain myelocariocytes and absolute count of different generation cells. Peripheral blood morphological examination was conducted by means of standard methods. The results of the experiments showed that irradiation at a dose of 9,0Gy causes bone marrow cell devastation of rats in the first hours after irradiation, achieving minimal rate to the second day. So, total number of cells in bone marrow in 48 hours decreased by 87%. In a group of animals that were injected api-phyto extract 10 days before irradiation a total number ofbone marrow at the period of first reaction for irradiation exceeded this rate of irradiated control group by 33-41%. This regularity was illustrative for rates of peripheral blood leukocytes, erythrocytes, platelets; in similar percentage and anti radical defense of organism in general. Parallel biochemical studies of blood serum of irradiated on the back of acceptation of api-phyto extract animals illustrated that hemoprotective effect realized by neutralization and detoxication of toxic radicals-radiotoxins, causing interphase (apoptic) death of myelocariocytes of bone marrow.

Keywords: radiotoxic products, hematopoiesis, radiation sickness, irradiation, pectin contain extract, natural antioxidant, lipid free-radical and per- oxidation.

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INTRODUCTION

Hematopoiesis correction of radiated animals is actual task of modern radiobiology as radiolesion depresses the process of cellular turnover and organism survival depends on speed of hematopoietic tissue regeneration. One of gene protective (anti-adaptogen) mechanisms of organism is preventing lipidfree-radical and per-oxidation, limitation of radiotoxic products creation of reaction, mobilization of bio-membranes and cell metabolism structure functional condition that possible with injection of substances having the ability to antiradical and antioxidative activity. Particularly, this is superoxide dismutase (SOD), catalase (CAT), glutathione reductase, carotenoids, flavonoids and other substances controlling and correcting free-radical processes by means of blocking of reactive oxygen and hydrogen molecules. It is possible to use successfully in clinical practice as radioprotective drug.

It is effectually to find out the causes of big variability of biological object radiosensitivity allowing to actively applyin clinical practice natural antioxidant as a radioprotective drugs that would be applied not as pure ferments or vitamins but as polybiocomponent formulation from aqueous, ethereal and ethanol extracts of phyto- and zoogamous origin.

Works in that direction are carried out constantly and it is known a lot of different pharmacochemical and biological products possessing radioprotective effect upon marrowy form of acute radiation sickness oflaboratory animals. In our research we set goal to study pectin containing extract influence on clinic and hematological condition of hematopoiesis and pro-oxidant and antioxidative status of irradiated animals.

MATERIALS AND METHODS

Experiments were carried out on 90 white rats with live weight of 180-210 g. divided into 4 groups: intact (1), irradiated (2), api-phytoextract + irradiation (3), irradiation+apy-phytoextract(4). The animals were exposed to single gamma irradiation on the setup “Puma” at dose of 9,0Gy. During 10 days before irradiation (the 3 group) and during 10 days after irradiation (the 4 group) animals were injected 10% api-phyto extract with water or feed at dose of 5-7,5 ml/kg.

The animals were dead with etherization and in the dynamic researched total number of myelocytes in bone marrow of femoral bone. In cell smear of bone marrow, we counted percentage of certain myelocariocytes and absolute count of different generation cells according to E.A. Zherbin and A.B. Chukhlovin [1]. Also mass of thymus was researched. In blood plasma and erythrocytehemolysate determined contain of lipid preoxygenation products- MDA according to G.Stark [10], antioxidant enzyme activity: CAT according to I.K.Kolomiyitseva [2] and SOD according to I. Fridovich [3]. Peripheral blood morphological examination was conducted with standard methods. Received numerical material was processed using variation statistics method by Student’s t-test with the use of application program package Microsoft Excel (2000).

RESULTS OF RESEARCH

Considering that the main aim of radical attack in radiation stress is hematopoiesis system changes ofblood system under the action of medication– api-phytoextract were investigated.

The results of the experiments showed that irradiation at the dose of 9,0Gy causes bone marrow cell devastation of rats in the first hours after irradiation achieving minimal rate to the second day. So, total number of cells in bone marrow in 48 hours decreased by 87%. In the group of animals that were injected api-phyto extract 10 days before irradiation, the total number of bone marrow at the period of initial reaction for irradiation exceeded this rate of irradiated control group by 33-41%. This regularity was illustrative for rates of peripheral blood leukocytes, erythrocytes, platelets; in similar percentage and for condition of pro-oxidant and antioxidant system with oral and parenteral drug administration of api-phyto extract (Charts 2 and 3).

Presented data in the Chart 1 illustrate that hemotoxic effect results in arrest of hemopoiesis with oppression of all basic bone-marrow hemopoiesis lineage : erythroid, neutrophilic and lymphoid. So, irradiated animals group after 10 days after irradiation the number of bone marrow cells was 1,61times less($P<0,01$), neutrophilic cells - 1,73 times less($P<0,01$) and lymphoid cells- 2,50 times less($P<0,001$) than intact animals

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had. These changes of bone marrow resulted in decrease of hemoglobin (1,05 times),number of erythrocytes (1,12 times), neutrophilic(1,91 times), P<0,01 in peripheral blood.

It was noticed hemoprotective effect on blood-forming in both groups of rats that received the drug both before and after radiation thanks to injection of api-phyto extract. So, in bone marrow of rats that injected api-phyto extract 10 days before irradiation the contain of myelocaryocytus exceeded irradiated control in 1,25 times, neutrophilic cells-1,57 times,lymphoid cells of bone marrow -1,74 times (P<0,01).

Survival growth of irradiated rats that got parenterally the drug of api-phyto extract normalization effect of hematologic state of white rats was observed (chart 2).

For the purpose of numeric data reduction here we present only the data of research on 3,7,14 experimental days i.e. periods of development and peak of height acute radiation sickness when irradiation hemotoxic effect gradually decrease to the end of experiment though researches were carried out in dynamic of (3, 7, 14, 21 and28) days.

Chart 1: Hemic system values (hemopoiesis) of white rats in 10 days after irradiation and treatment and prophylactic administration of api-phyto extract

Index	Group			
	control	irradiated	irradiated.+treatment 10 daysbefore irradiation	irradiated+treatmentduring 10 days after irradiation
Total number of cells in bone marrow of femoral bone $x10^6$	27,5±1,8	17,8±0,8*	22,2±0,9	21,9±0,98
Total number of erythroid cells in bone marrow of femoral bone. $x10^6$	5,53±0,44	3,43±0,34*	4,35±0,26	4,31±0,35
Erythrocytes, $x10^{12}$/l	9,1±0,4	8,3±0,14	8,9±0,31	8,7±0,41
Blood hemoglobin content, g/l	112,4±4,8	107,1±2,9	111,5±3,1	110,3±2,8
Total number of neutrophilious cells in bone marrow of femoral bone. $x10^6$	15,45±1,8	8,92±0,22**	13,97±0,78	12,44±0,57
General blood count of neutrophilious. $x10^9$ /l	2,16±0,35	1,13±0,75**	1,79±0,38	1,67±0,41
Total number oflymphoidcells in bone marrow of femoral bone$x10^6$	4,44±0,41	1,77±0,31**	3,09±0,85	3,01±0,78
lymphocyte$x10^9$ /l	4,36±0,57	1,67±0,5**	3,61±0,95	3,57±0,77
Thymus mass, mg	46,3±3,2	30,1±2,5*	41,9±2,7	40,8±3,3
Thymus mass coefficient, mg/100g	0,23±0,05	0,15±0,03	0,21±0,05	0,20±0,01

As chart 2 illustrates lethal dose irradiation of rats makes hemotoxic effect. To the 3 day after irradiation number of leukocytes went down by 2,3 times(P<0,01) that progressed to 14 day when its number came short of control to 3,4 times(P<0,001) and till animals death this tend saved. Applying of api-phyto extract prevented to catastrophic lowering of leukocytes of irradiated animals.

Chart 2: Hematological factors of white rats with irradiation and api-phyto extract application

Group	Duration of experiment, days	Erythrocytes, $*10^{12}$/l	leukocytes, $*10^9$/l	Hemoglobin, g/l	Hematocrit, %	Protein, g/l
1.	3	7,40±0,48	11,58±0,31	126,3±6,8	36,34±0,85	71,8±3,1
	7	7,42±0,52	9,89±0,53	124,7±5,2	36,27±0,61	70,9±3,8
	14	7,38±0,56	9,77±0,63	133,3±4,4	36,14±0,45	69,8±2,9
2.	3	7,05±0,27	3,74±0,34*	112,5±4,9	32,24±0,68	62,9±2,3
	7	7,07±0,44	3,41±0,28*	106,6±2,3	30,3±0,72	60,3±3,6
	14	6,03±0,38	3,09±0,37*	101,2±2,9	29,09±1,09*	57,8±4,1

3.	3	7,23±0,33	5,04±0,48	116,7±5,0	35,3±0,27	69,0±2,7
	7	7,19±0,23	5,76±0,43	118,3±4,8	35,87±0,31	70,2±4,5
	14	7,25±0,43	6,89±0,22	120,3±3,9	36,02±0,53	70,4±3,8
4.	3	7,16±0,22	4,96±0,31	114,1±5,2	34,84±0,35	66,1±2,2
	7	7,02±0,31	4,85±0,44	117,3±2,8	35,34±0,42	69,3±2,4
	14	7,21±0,18	5,97±0,12	119,7±3,1	35,92±0,38	69,8±3,7

So,on the14th day after irradiation lowering of leucocytes of animals in the 3 group that were given the drug during 10 days before irradiation was 30% and 39% in the 4 group. By the end of experiments (28 day), peripheral blood leukocytes of irradiated rats on the back of api-phyto extract administration, un authentically, came short of control with 89,7%(the 3 group), 83,5 % (the 4 group). Hemotoxic effect of irradiation resulting in leukopenia, erythropenia, hemoglobulinemia lowing the protein andhematocrit, and oral acceptation of the test preparation made hemoprotective effect preventing blood values lowering.

On the back of treatment and prophylactic injection of the drug decreasing the clinic manifestations of acute radiation sickness appeared tend to be lowering of POL, MDA concentration in blood plasma (Chart 3). At the same time as reaction on therapy lowering of the mark of animals of the 4 group was more evident than in the 3 group. Treatment applying of api-phyto extract had clearer anti-oxidative effect. Anti-oxidative status effect examination of treated and prophylactic animals confirmed it.

Under the drug effect concentration of antioxidant enzymes of irradiated animals was 1,3 times lower (SOD) and 1,13 times (CAT) in comparison with the control (P<0,05).

Chart 3: Content MDA SOD CAT in blood plasma of api-phyto extract irradiated white rats in 10 days after irradiation

Группа	Content		
	MDAmcmol/l	SODu/l	CATmcat/l
The intact	0,19±0,02	1,66±0,03	25,03±1,47
Irradiated	0,65±0,05	0,87±0,06*	12,61±0,38*
Irradiatedandreceiving api-phyto extract 10 days before irradiation	0,29±0,04	1,22±0,48	19,84±2,52
Irradiatedandreceiving api-phyto extract during 10 days after irradiation	0,27±0,16	1,24±0,38	25,07±3,04

Watchful waiting for clinical condition over lab rats' results showed that animals that were given the drug orally (powder and liquor of the extract) differed from the control and only irradiated animals in increased appetite, less prominent loss of live weight, responded to external stimuli appropriately, had cleaner look,coat of wool took bloom.

Therefore, hematological marks confirmed the data hemopoeisis, clinic and antioxidative activity of blood serum received from study of radioprotective effect of api-phyto extract protecting from radiation death 50-80% of lethal irradiated animals that got the preparation orally.

As known, lethal dose radiation results cellulated devastation of blood pool via devastation of bone marrow of rats for the initial hours after radiation reaching the lowest values to the 2nd day. In our researches general cells number of bone marrow decline by 87% and was only 15% of initial level for 48 hours after irradiation. As of the 3d day slowly hematopoiesis recovery that did not achieve the control level up to the end of experiment was happening. Sharp down of bone marrow cells at the period of initial reaction on irradiation fit in the scheme of mitotic cell death: pause in entry into mitosis, creation of nonviable giant cells, death of the first division, death of the rest divisions. Pause in cell division cause is destruction of substances stimulating mitosis, disruption of cell membranes permeability, disturbance of nucleonic acid synthesis, structural chromosomal damage. Many authors [4,7,8] think that mass death of bone marrow cells at an early stage (10-12 h after irradiation) is interphase, i.e. occurring before the next division.

In pathomorphological term interphase death of myelocariocytes shows as general bone marrow ″necrosis″. Interphase death symptoms are observed most clearly in pathology of nuclear cell structure pyknosis(homogenization) of nuclear material, chromatin decay, cytoclasis with creation of so called apoptotic corpuscles [5]. Frequency of bone marrow cells with pyknotic nucleuses at the early post-radiation period depends on exposure dose [6-10].

In our experiments hemopoiesis picture of rats at the period of priming reaction on irradiation was characterized by profound leucopenia (leukocytes number was $1,6 \cdot 10^9$/l). Considerable reduction of total number of leukocytes was caused by evident leucopenia. Erythrocyte number at this period dropped to $3,45 \pm 0,34 \cdot 10^9$/l and thymus weight and its mass coefficient dropped to 30,1±2,5 mg и 0,15±0,03 mg/100 g against 46,3±3,2 mg and 0,23±0,05 mg/100 g of intact animals consequently. On the 10th day after irradiation animals of this group had clearer lymphopenia, erythropenia, thrombocytopaenia. Figures received because of external exposure effect of dose of 9,0 Gy in our experimental were to control the 3 and 4 group of animals that were injected api-phyto extract before and after irradiation. Hemo- and mieloprotective effect of the drug resulted in reciprocal increase of erythrocytes (by 7%),neutrophils (by 58%),lymphocytes (by 26%), hemoglobin (by 3%) in peripheral blood with synchronistic growth of mass coefficient of thymus 40% more (P>0/05).

So, injection api-phyto extract as before (10 days) so after (during 10 days after) lethal irradiation influenced as radioprotector realized by activation of hemopoietic microenvironment elements early hemoethic (progenitor) cells and microstroma cells.

Among numerous hypothesis and theories explaining mechanisms of radiation damage, free-radical theory [7] explaining the role of oxygen in radiobiological «oxygen effect» takes important place. Herewith, it's brought into sharp focus that irradiation with ionizing emission produces sharp activation of lipid preoxygenation and superoxide dismutase (SOD) prevents this effect [3,6]. Then slight increase in free-radicals can cause chain process peroxidation in irradiated bio-objects. Lipid structure changes, viscosity changes and mobile at aqueous phase lipid radio-toxins that trigger the mechanism of radiation-induced apoptosis liberate. Parallelly oxidative deamination, decarboxylation, hydroxylation peptide bone break, aroma amino acid modification, oxidation-reduction of phenols happens in proteins that determine emergence of another class of high toxic compounds–quinoid radio-toxins. On the body level besides the fact that ionizing radiation as stressor activates pituitary and causes intense synthesis of adrenocorticotropic hormone (stress hormone) the process is worsened by formation auto-radio-toxins of different classes from proper cells and, consequently, now the picture of early acute radiation damage can be considered as physically not perceptible stress-reaction on latency tissular at the initial period.

New tasks appear for radiobiologists in the search and estimation of antiradiation protective substances and methods such as limitation of radionuclides intake, protection of cells and tissues (substitutive precaution and therapy),elimination(clearance) of radionuclides and antioxidant defense from high toxic free radicals. At that, the prime determinant factor of concentration of toxic substances (free-radicals) in cells is cooperative work of key antioxidant ferments- superoxide dismutase, catalase, glutathione peroxidase making peroxide detoxication by means of interception deactivation and modification of free-radicals [8].

Consequently, the use of natural products (bio-antioxidants) is incomparably preferable, as they are complexes of biologically active substances that are similar in nature to endogenous bioregulators and adaptogens taking biologically adequate correlative effect on the functional state of the organism on the back of stress factors.

Api-phyto extract influence on condition of hemopoeisis of irradiated animals is obvious. In mechanism of radioprotective effect, its ability to aggravate processes of blood-forming tissue recovery is unique. So, in the group of animals that were injected api-phyto extract before and after exposure total number of bone marrow at the period of initial reaction for irradiation exceeded this rate of irradiated control group by 33-41%. This regularity was illustrative for rates of peripheral blood leukocytes, erythrocytes, platelets in similar percentage. It is possible to assume that api-phyto extract reverses accumulation of radio toxins that cause mitotic and interphase death of bone marrow cells of irradiated animals and has ability to protect substances, stimulating mitosis prevent disruption of cellular membrane permeability and disruption of nucleic acid synthesis.

Precaution, treatment and correction of activation of radio-induced free-radical radiopathology allows to weaken and change all following stress factors forming adequately to the radiation effect of compensatory adaptive reactions with the help of an herbal and animal composition in the form of a dry and liquid dosage form for oral use, having both antioxidant, adaptogenic, hemoprotective and immunomodulating and radioprotective properties.

CONCLUSION

The results of carried out researches show radioprotective effect of api-phyto extract that appears in correction of hemopoeisis of irradiated animals through of neutralization and detoxication of toxic radicals-radiotoxins causing interphase (apoptic) death of myelocariocytes of bone marrow and also correction of the pro-oxidant and antioxidant system of the organism in the conditions of radiation oxidative stress, preventing the accumulation of oxidative macromolecules in the irradiated organism by using a multicomponent pectin-chitin-containing drug neutralizing the effects of oxidative stress.

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