

The comparative analysis of the preoptic lateral, arcuate and paraventricular nuclei monoaminergic systems in ontogenesis showed a great decrease of monoamine fluorescence in the preoptic area nuclei of the aged animals and practical absence of changes in the hypothalamic arcuate and paraventricular nuclei. In the paraventricular nucleus of prenatal stressed female monoamine fluorescence was rather low during the all ontogenetic periods.

Key words: prenatal stress, ^3H -estradiol, monoamines, hypothalamus.
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**THE ASSESSMENT OF BIOEFFECTS OF THE SPECIAL HERB
AND MINERAL COMPLEX AT LONG TERM EXPOSURE
TO INCORPORATED LOW LEVEL RADIOACTIVE
CAESIUM-137 AND STRESS**

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Key words: radioprotectors, nutritional supplements, Caesium-137, internal radiation, lipid peroxydation.

Abstract. The assessment of the biological action of the herb and mineral origin nutritional supplement complex produced by Mineral Resources International (USA) was experimentally conducted on laboratory rats which were under the long term exposure to incorporated low level Caesium-137 isotope (^{137}Cs) radiation at 1200 Bk and immobile stress.

The influence of the studied complex on accumulation and elimination of ^{137}Cs , the level of hormone activity in blood, lipid peroxydation processes in endocrine organs, hypothalamus, as well as hepar and lungs was detected. Besides the morphofunctional state of the brain and endocrine organs was studied.

It was revealed that the tested complex of nutritional supplements has the aptitude to decline ^{137}Cs accumulation. The normalization of the lipid peroxydation processes and the prevention of rough dystrophic and destructive alterations development in endocrine organs both the blood microcirculatory surfaces and secretory cells in its turn proves radioprotective properties of this nutritional supplement complex (registered in Ukraine, patent № D 000128 applied for).

Introduction. As the consequences of the Chernobyl catastrophe include besides external ionizing radiation the constant intake into human body of the substances with the low radioactivity for a very long period and presence of the psycho-emotional factors it exhibits the real threat to health [1,2]. These circumstances have limited the implementation of classical sorbents, because the latter can remove the whole line of essential nutrients from the organism. That is why application of sorbents is possible only during short time and with simultaneous supplementation of the special diets directed to restore the abnormal biochemical balance. In the real situation it is rather reasonable to apply the preparations which have the properties to eliminate mainly radioactive isotopes and could be taken for a long time. From this point of view very

attractive are the properties of the nutritional supplements based on natural minerals and herbs promoting to normalize the state of the majority systems and functions [3]. That is why one of the purposes of the present research was to assess the probable influence of the special nutritional supplement complex produced by Mineral Resources International on the processes of accumulation and elimination of the radioactive Caesium (^{137}Cs) from organism. This isotope was chosen because its share is 94% of the whole bulk of accumulated radionuclides in the organism [4]. As it is known one of the most affected to low dose ionizing radiation is such an important regulatory system as hormonal as well as the structure of neuroendocrine organs [5]. The data from literature give evidence that impair of the endocrine system function results in changes of other systems bound with it, which form together an integral complex, preventing the adaptation of organism to unfavourable environmental factors [6,7]. Taking into consideration the core of the hypothesis substrate level of the organism defense worked out by Е.Б. Бурлакова, Л.Н.Шишкина (1982) [8] which meets to that lipid constituents of membrane serve as the main physiological regulator of the lipid peroxydation processes. At the exceeding the lipid peroxydation from normal membranes enrich themselves with the forms of phospholipids (sphingomieline, phosphatidylcholine) resistant to peroxydation. It leads to retardation of the lipid peroxydation speed and the decrease expenditure of the antioxidants. On the contrary, the decrease of the phospholipids causes the elevation of the light oxidized fractions of phospholipids (phosphatidylserine, phosphatidylinositol) containing much higher amount of unsaturated fatty acids, that, in its turn, increases the speed of free radicals formation, but for all that the abundance of antioxidants was utilized. Besides, the changes of the membrane constituents comes to alteration of membrane-related enzymes activity including such an important for regulatory processes of the adenylatcyclase messenger, as cyclic adenosine monophosphate releasing neurohumoral regulation of metabolism (Sutherland E.W., Robinson I.A., 1966). Thus, the availability of the substrate regulative universal biologic phenomenon gives the basis to the free radical formation to be balanced by the essential nutrient compositions having radioprotective and membranestabilising properties at the long term exposure to low level irradiation. From this point of view, the influence of the special nutritional additive complex on the level of hormones in blood, the lipid peroxydation processes in endocrine organ tissues as well as their morphofunctional state was studied.

Subjects and methods. The researches were carried out on 80 white mature nonlinear male rats with body weight of 150-200 gr. at the begining of the experiment. Three equally numbered experimental groups were formed. Animals of the first group were exposed to the 1200 Bekkerel (Bk) of ^{137}Cs daily per each for a month and to the influence of immobile stress, weekly (4 times totally). The second group at the same model of exposure received the special nutritional supplement complex, consisting of the Nutritional Supplement formulated for the Adults of Ukraine, plus Stress-X, plus CellEnergy (additive 1) in a dose estimated per kg of body weight corresponding to the company proposal for regular use of every nutritional supplement. The third group of intact animals served as a control. The animals of all experimental groups were in identical conditions at the air temperature of 18-20°C, relative humidity of 55-60%. The tested and control animals were under identical forages.

On the accomplishment of the experiment the rats were guillotine decapitated under ether narcosis. The fasting blood samples were obtained. The plasma was separated from the EDTA anticoagulated blood by centrifugation of 4000 g for 10 min. All blood samples were labeled, stored and collected to the freezer at $t - 25^\circ\text{C}$. The content of the adrenaline in plasma was detected by the physicochemical method [9], of the corticosterol in serum - using the fluorometry [10]. The content of triiodothyronine (T_3), thyroxine (T_4), insulin in blood serum was found by the radioimmunassay method [11].

The tissues of the hypothalamus, the hypophysis, the adrenals and the thyroid were removed during 5 minutes after the decapitation. The Malone dialdehyde (MDA) level in

studied organ tissues was detected by Ю. Ф. Владимиров, Арчаков А.М. (1972) method [12].

For detection of the hormones and MDA level the following equipment: centrifuges VAS - 125, "Becman" K 26 D, «Coolspin 2», PS-6, OPN -3, OPN - 8, lab scales VRL - 200, torsion scales VT - 50, electron scales ER - 182 A and FX - 320; spectrofluorometer «Hitachi F-4000», photocolimeter KFK-2, ionometer I-120, pH-meter V-629, hamma-counter «Trac - 1191» were used.

Light microscopic examination of the series of semithin sections, received from electron microscopic blocs embedded Epoxy resin was also conducted. Sections were obtained on ultramicrotome - 1R5 "Reichert" and after painting with methylene blue-pyronine were looked through light microscope. Ultrathin sections by thickness of 600Å were cut on a ultramicrotome 1R5 "Reichert". Ultrathin sections were extrapainted as to Reingolds and were looked through electron microscope EM-400T "Philips" (Holland).

The ^{137}Cs was used for the internal irradiation of the tested animals. The latter were catered with grinded bread, put in the solution of ^{137}Cs chloride at the rate of 1200 Bk. daily per animal. The treatment was carried out daily for 30 days. The total duration of the experiment lasted 35 days. The detection of dose was carried out on the hamma-spectrograph type LPC 4950 with BOEG - 10V of system «Nokia» (Finland). A special phantom modeling the body shape of the animal in order to estimate the coefficient, depending on geometry of the rat body was used for measuring its hamma-activity. Special powder containing ^{137}Cs at the moulding of this phantom was used. The hamma-activity measurement was conducted every two weeks, totally two times during the experimental period. Additionally, the animals were exposed to the influence of immobile stress weekly in Kohan camera. Such model is rather widely used in physiological researches, because of simplicity of technique and the opportunity to precise dosimetry [13].

The processing of obtained data was carried out according to the programs "Excel-7" and "Statgraphics" [14] ($p < 0.05$ was considered significant).

The results and discussion: The data of specific activities of animals receiving and non receiving the special nutritional supplements (additive 1) in dynamics are reflected in Table I.

The investigation has shown that the hamma-activity level significantly differed in three groups for all period of the observation, and have witnessed that the studied dose of the additive 1 posses the properties to diminish the accumulation of ^{137}Cs in the body of tested animals.

The study of the hormone activity testified that the treatment of the rats with radioactive ^{137}Cs and the stress modeling for 30 days has resulted in significant increase of the adrenaline and corticosterol content in the blood of animals. But the supplementation with the additive 1 no markedly induced on their levels in comparison with the data of the model lesions. However, additive 1 regulatory influences on the lipid peroxydation processes in majority of studied organ tissues. But complete normalization of MDA concentration was only found in the adrenals (Table II).

Nevertheless, the obtained data showed, that the action of ^{137}Cs and stress caused the significant decrease of the Malon dialdehyde (MDA) content in the testes, the adrenals, the thyroid, the hypothalamus, and the hepar. There are no significant changes only in the pancreas and the lungs.

The decrease of the lipid peroxydation level observed in experiment can be explained from the point of view of turning on the compensative mechanisms at the level of the physicochemical system of the lipid peroxydation regulation [15,16]. The essence of the compensative response comes to the modification of the membrane phospholipid components in such way that to lessen the content of the fatty acids with unsaturated double bonds, while the lipid peroxydation processes lose their substrate security and their intensity decreases. The changes shown by electron microscope analysis have convertible characters and come to normal at the discontinuance of ^{137}Cs intake. If the irradiation of animals continues the membranes undergo the line of destructive reconstructions accompani-

Table I. The comparative analysis of specific radioactivities of the animals which were under a long term treatment with the complex of special nutritional supplements (additive1) at the exposure to radioactive Caesium-137 and stress in dynamics ($x \pm Sx$).

Condition of the experiment	Dynamics of specific radioactivity (Bk/g) after 2 weeks of treatment n=20	Dynamics of specific radioactivity (Bk/g) after 4 weeks of treatment n=20
Caesium-137 +stress introduction	2,43 ± 0,38	4,09 ± 0,68
Caesium-137 + stress + additive1 introduction	0,86 ± 0,09*	2,48 ± 0,24*

* $p < 0.05$ with reference to corresponding term of the model lesion affect.

Table II. The comparative analysis of the special nutritional supplement complex (additive1) effects on the Malon dialdehyde (MDA; nmol/mg, protein) content in the endocrine organs of rats, which were under exposure to chronic Caesium-137 incorporation and stress (1 month) $x \pm Sx$.

Tested organs	Control n=20	Caesium-137 +stress n=20	Caesium-137 +stress +additive 1 n=20
tested	413.10 ± 50.30	120.80 ± 11.90*	216.3 ± 10.2***
adrenals	191.2 ± 18.8	38.90 ± 4.59*	202.2 ± 8.3***
pancreas	172.30 ± 15.50	298.30 ± 6.60*	113.4 ± 5.1**
thyroid	237.30 ± 30	45.3 ± 6.70*	116.3 ± 14.3***
hypothalamus	655.40 ± 25.70	453.10 ± 15.70*	248.8 ± 21.0***
hepar	440.10 ± 40.16	167.30 ± 32.60*	272.2 ± 15.4*
lungs	221.10 ± 24.80	203.30 ± 13.50	132.1 ± 32.2

* $p < 0.05$ with reference to control

** $p < 0.05$ with reference to Caesium-137 +stress

ed by the lipid peroxydation activation. The scarcity of the lipid phase of membranes caused by the irradiation depriving membranes of their turnover leads to changes of kinetic parameters membranes related enzymes, such as potassium-sodium adenosinetriphosphatase, what can be the reason of impoverishing the cell receptors on membrane surface of endocrine organs.

The conducted histologic and electron microscopic researches of the animals exposed to incorporated irradiation of ^{137}Cs and immobile stress for a month showed that in all studied endocrine organs and hypothalamic region of brain different degree dystrophic and destructive alteration appearances both from side of microcircular vascular surfaces and from neurosecretory cells of the hypophysis, the adrenals and the thyroid were found.

Nevertheless the decrease of secretory activity of these cells accompanied by the decrease of their energoproducing function because of mitochondria destruction, especially in adrenocorticoocytes of adrenal cortex as well as the suppression of the proteinsynthetic function to definite extend due to reduction of the number of free and-bound ribosomes and redistribution of the nuclear chromatin into cell nuclei were observed.

Histologic examination of the hypothalamus and endocrine organs (the hypophysis, the adrenals, the thyroid) of animals being catered with the additive I at the combined exposure to radiation and chronic stress showed that the slight oedema phenomenon decrease astrocytes processes of neuroglia adjoining to microvessels was observed in the hypothalamic area and that is the result of the lessen of perivascular swollen. In studied endocrine glands the similar picture of the normalization of structural microvessel wholeness was present as well as significant decrease of the oedema of cellular elements of these organs. However the most proved data concerning the efficacy of studied additive I were obtained at electron microscopic examination. Received results convincingly showed that the perivascular oedema phenomenon of astrocytes neuroglia at catering rats with the additive I were considerably reduced in a month, which sharply influenced on the structural wholeness of endothelial lining in the main mass of microvessels. Even hyperplasia of marginal part of endotheliocytes featured the increased number of ribonucleic granules and intensification of morphofunctional status at the background of micropinocytous vesicles raise in number were observed.

The next important factor of the additive I action lies in intensification of the morphofunctional activity of neurosecretory cells of the hypothalamus the adeno-hypophysis, the adrenals, and the thyroid.

Thus, after chronic action of internal radiation and stress the obvious lowering of hypothalamus neurosecretory cells was seen. On the contrary after catering with the additive I quite opposite activation in the part of neurosecretory cells took place and the increase of secretory activity in the part of somatotroph and thyreotroph cells and majority of adrenocorticotrophocytes in the hypophysis was revealed, too.

In comparison with ultrastructural changes in the hypophysis and the hypothalamus there is normalization morphofunctional status in the adrenals of catered animals in the whole, including both the cortical and medullar matters. The most vivid activity was observed in fasciculata cell zone - spongiocytes. The number of lipid includes at the background of hypotrophia of majority mitochondria and the increased picture intensity of endoplasmic reticular rete in these cells considerably grown in comparison with affects of radiation.

Rather expressed hypertrophia and hyperplasia of marginal part of endotheliocytes accompanied by the intensification of micropinocytous activity and appearance of intracellular organelli and first of all the great amount of free ribonucleic granules in cytoplasm, showing the growth of proteinsynthesizing function of these cells is observed in endothelium of peritubular capillaries of the thyroid gland.

However, a lot of cells remained in the state of dystrophy and the lowering of secretory activity occur both in the thyroid and all studied endocrine organs and hypothalamus which is the result of radioactive damage. It is necessary also to underline that the dominated mass of cells with high secretory activity is located alongside the functionally active

microvessels, while the secretory cells with the low activity are concentrated nearby the extended and injected microvessels. So to say there is the correlation between activity of the secretory cells and state of intraorgan vessels.

Thus, the use of the special combination of nutritional supplements in animal forage brought to the normalization of the blood microcirculatory system in all studied tissues and first of all in areas hypothalamic nuclei, that undoubtedly leads to activation of neurosecretory cells.

The influence of additive 1 on functional status of adrenals was the most demonstrative and exhibits the increase of lipid includes number in cytoplasm of cortex puberozona that witnessed the hyperproduction of corticosterol synthesis.

Conclusion: The effects of the special herb & mineral origin nutritional supplement complex A Special Ukrainian Adult Herb & Mineral Nutritional Supplement, Stress-X, CellEnergy (additive 1) daily administered to mature white male rats under the model exposure to ^{137}Cs at 1200 Bk and stress for a month were studied.

It was detected that:

1. The tested complex of nutritional supplements has capabilities to decrease ^{137}Cs accumulation.

2. It doesn't exercise regulatory on hormone activity but causes the normalizing influence on the lipid peroxydation processes in cell membranes of the tests, the thyroid and the hepar and the complete normalization takes place only in the adrenal glands.

3. The introduction into the diet of rats of the special complex prevents the development of rude dystrophic and destructive changes both in blood microcirculatory surface and in secretory cells of endocrine organs, which undoubtedly can be considered as the radioprotective effect of the tested nutritional supplements.

Thus the studied nutritional supplement complex produced by the Mineral Resources International possess the capabilities to diminish ^{137}Cs accumulation in organism of tested animals as well as membranostabilizing properties (registered in Ukraine, patent № D 000128 applied for).

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**ОЦІНКА БІОЛОГІЧНОЇ ДІЇ КОМПЛЕКСУ СПЕЦІАЛЬНИХ
РЕЧОВИН МІНЕРАЛЬНО-РОСЛИННОГО ПОХОДЖЕННЯ
В УМОВАХ ДІЇ МАЛИХ ДОЗ ІНКОРПОРОВАНОГО
ІЗОТОПУ ЦЕЗІЮ-137**

Резюме. В експерименті на лабораторних щурах була проведена оцінка біологічної дії спеціального комплексу харчових домішок, виготовлених фірмою Mineral Resources International (США), в умовах довготривалого надходження радіонуклідної контамінанти ізотопу цезію-137 (1200 Бк щоденно) і хронічного стресу. Досліджувався вплив комплексу, що вивчався на накопичення та елімінацію цезію-137, рівень гормональної активності, процеси перекисного окислення ліпідів у тканинах гіпоталамусу, сім'яників, наднирників, підшлункової залози, в печінці і легенях. Крім того, вивчався морфофункціональний стан ендокринних органів.

Було встановлено, що спеціальні харчові домішки мінерально-рослинного походження сприяють зменшенню всмоктування або підсилюють елімінацію ізотопу цезію-137, визначена їх регулююча дія на процеси перекисного окислення ліпідів у мембранах клітин сім'яників, печінки, щитовидної та підшлункової залоз і, особливо, в наднирниках. При цьому попередження розвитку грубих дистрофічно-деструктивних змін в мікроциркуляторному судинному руслі і клітинах ендокринних органів, за даними гістологічних та електронномікроскопічних досліджень, підтвердило радіопротективні якості комплексу нутрієнтів.

Таким чином, експериментальне дослідження комплексу харчових домішок у складі A Special Ukrainian Adult Herb & Mineral Nutritional Supplement, Stress-X, CellEnergy визначило його радіопротективну дію, а також мембраностабілізуючі властивості (zareєстрований в Україні, сертифікат № Д 000128).

Ключові слова: радіопротектори, харчові домішки, цезій-137, внутрішнє опромінення, перекисне окислення ліпідів.

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