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INVESTIGATION THE SELENIUM-COMPRISING CHICORY PHYTOCOMPOSITES AS RADIOPROTECTOR AGAINST ACUTE AND CHRONIC IRRADIATION

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Abstract: The radioprotective properties of selenium-comprising chicory phytocomposites (SECP) have been studied in a series of animal experiments. The prospectively of selenium as a basic material for food supplements and functional nutrition products for protection against acute and prolonged irradiation is demonstrated. The study demonstrates the radioprotective effect of the SECPE at both acute and prolonged irradiation exposure. This effect is based on the high biological activity of the SECPE which stimulates, regulates, and restores the psychoneuroimmune system and the non-specific resistance mechanisms. In our opinion, the SECPE radioprotective effect is most expressed at acute irradiation exposure. Based on the SECPE, we have elaborated a dietary food supplement with biological activity – "Selenium-S", which is promising both being used *per se* and for functional product development with the purpose of psychoneuroimmune regulation improvement including cases of radiation damage and requires further investigation of use peculiarities.

Keywords: selenium-enriched chicory, phytocomposite extract, acute and prolonged radiation, radioprotector

1. Introduction

The population health level depends significantly on the natural and artificial irradiation factors. A substantial component of the negative environmental influence on the human body now is the excessive ionizing irradiation exposure which can be a consequence of the following: contacts with natural low-intensity irradiation sources situated in certain geological inhabited areas; working in nuclear power stations and radiological/X-ray laboratories; technogenic disasters with growing occurrence at Chernobyl as well as Fukushima [1-4]. Nowadays, dietary food supplements (DFS) or functional foodstuffs with defined properties introduced into the diet are believed to be the optimal option for human body protection against the complex negative environmental factors influence including irradiation [5-7]. Thus, the search for a novel vegetable material to be used as a radioprotector in the case of acute and, especially, chronic irradiation is of an outstanding urgency and timeliness. As shown in our recent investigation influence radiation on biological organism depend on a lot of different stress factor [8-9]

One of the most widely used vegetable irradiation adaptogens (radioprotectors) is the chicory [10-12]. It is known that the combination of vegetable-derived biological complexes and trace elements/vitamins can increase the body impact of the former significantly [10-12]. Our preliminary investigations have shown the prosperity of further study of naturally selenium-enriched chicory stuff and its phytocomposite derivatives as a means of positive influence upon the psychoneuroimmunoendocrine regulatory system [13].

The study purpose was to investigate the possible radioprotective effects of the selenium-enriched chicory phytocomposite extract (SECPE) in acute and prolonged radiation exposure.

2. Materials and methods

The study was carried out at the Microbiology, Virology and Immunology Department of Maxim Gorky Donetsk National Medical University; Neurophysiology Research Laboratory of the National Academy of Medical Sciences of Ukraine; Commodity Research and Foodstuffs Expert Assessment Department of Mikhail Tugan-Baranovskiy Donetsk National University of Economics and Commerce; Cell Biology and Genetic Engineering Institute of National Academy of Sciences of Ukraine.

In the model experiments, there was 125 male outbred white rats with the body mass 170-200 g involved. The animals were distributed by 25 units to 5 groups: 2 main groups, 2 control groups, and 1 intact control group. The rats were kept with the observation of this species' usual requirements [14]. All the experiments met the demands of the "General Principles of Animal Experiments" adopted by the First National Congress in Bioethics (Kiev, 2001), the international principles of European Convention "For the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), and the law of Ukraine № 3477-ИМ "For the Protection of Animals against Cruel Treatment" dated by 21st February 2006.

The intact animals (group I) were kept on a usual diet and were not exposed to irradiation. The controls also got a common ration but they were exposed to acute (group II) or prolonged (group III) irradiation. The animals in the main groups obtained a diet enriched with SECPE and were exposed to irradiation: group IV underwent acute irradiation and group V was subject to prolonged irradiation exposure.

The SECPE was produced by the original technology developed in the Cell Biology and Genetic Engineering Institute of the National Academy of Sciences of Ukraine. The chicory phytocomposite (SECPE) was administered to the rats of the main groups orally with drinking water dosed 1.0 ml per animal daily for 3.5 weeks; after that, the rats were exposed to acute or prolonged irradiation in the half-lethal dose of 6.5 Gy. For the irradiation sessions, the Rokus-M #126 (a gamma-therapy apparatus) in the radiologic department of Donetsk Regional Oncological Centre was used. The acute irradiation exposure dosed 6.5 Gy was performed as a single session. The prolonged irradiation was carried out on alternate days during a month in the dose of 0.44 Gy per session so that the total dose amounted to 6.5 Gy.

The observing time for the animals after the acute irradiation was equal to 1 month while the rats which had undergone the prolonged irradiation course stayed under observation for 4 months after the discontinuation of the SECPE food supplementation in the main group. Prior to the experiment, after the SECPE administration and 1 month post irradiation, the animals have physically examined: the body weight, integument status (including mucosa, hair, and underfur) were estimated. At the same time, the blood and immunity status, as well as the grooming reactions, were assessed. The peripheral white blood cell count was measured [15]; leukocyte pools and their morphological changes were defined. The content of leukocytes and their pools was measured in absolute units (G/l); the leukocyte pools' morphology alterations were represented in relative units (%) [16]. The body auto sensitization level was registered according to the method of Slusarev A.A., Raksha-Slusareva Ye.A. [17]. The elements of the non-specific resistance mechanisms were studied by the parameters of biochemically conditioned bactericidal activity of neutrophilocytes using the nitro blue tetrazolium (NBT) test [18]. The grooming parameters were investigated as indicative signs of the higher nervous activity of the rats [19].

The data obtained were processed by the variation statistics methods using PC and appropriate program packages including «Statistica for Windows».

3. Results and discussion

Table 1 demonstrates the leukocyte total content and their pools in the animals of experimental groups prior to and after the ionizing irradiation exposure. According to the table, the control group II rats showed a considerable and significant decrease of peripheral blood white cell count with the diminishment of the neutrophilocyte, monocyte and lymphocyte content compared with the intact animals (group I) during the study and the specific normal range ($P < 0,05$). On the average, the rats in the IV (main) group which obtained SECPE prior to and after the irradiation exposure also demonstrated a significant reduction of the peripheral blood white cell count versus the initial parameters and the control group data, but these indices were still within the specific normal range lower limits. The white blood cell count in the IV group of animals (8.39 ± 1.28 G per l) was significantly higher than that in the II group (5.14 ± 0.07 G per l) ($P < 0.05$). The white blood cell count diminishment in these animals was mostly caused by the reduction of the neutrophil pool; nevertheless, the neutrophil content in the IV group was still higher than that in the II control group. The rats which obtained SECPE showed after the acute irradiation a significantly higher content of metamyelocytes, stab neutrophils, and eosinophils compared with that in animals of the II control group, the baseline levels in this group, and the intact rats. Monocyte content was virtually indistinguishable from the initial level, specific normal range, and the intact animals' data. Lymphocyte content in the IV group animals just tended to decrease staying within the normal limits; it significantly differed only from the intact group's level which was slightly increased. The animals of the III control group exhibited a slight tendency towards the decrease of the white blood cell count compared with the baseline level, the first group's data during the study, and the specific norm. In the III group, there was a significant reduction of segmented leukocyte content along with an increase of stab neutrophil level in comparison with the baseline data, intact animals' and specific control's levels ($P < 0.05$). A considerable but statistically insignificant increase in eosinophil content was detected. Monocyte and lymphocyte levels were within the baseline and specific norm limits.

The animals of the V group which were administered SECPE showed the white blood cell count within the normal range and similar to the baseline and intact group's levels. The neutrophil content was considerably higher than that in both control groups and the III main group, but still, it was below the baseline and intact group's levels ($P > 0.05$). A considerable and significant increase of metamyelocyte and eosinophil content ($P < 0.05$) exceeded that in the III group, while the stab neutrophil and monocyte level were raised insignificantly. The latter was caused by the great individual numerical variability of these leukocyte pools. The lymphocyte content in the III group animals was similar to the baseline and specific norm levels and was indistinguishable from that of the intact group.

After the acute irradiation, the cell dissolution count in the peripheral blood of the animals in the II (control) and IV (main) groups made up 27.50 ± 5.00 % and 26.80 ± 7.50 %, respectively, what significantly exceeded the baseline level of 5.00 ± 0.60 % ($P < 0.05$). The content of cells with membrane destruction after the irradiation exposure rose considerably and significantly from 3.50 ± 0.51 % to 26.1 ± 6.10 % in the control group and even more, up to 35.90 ± 8.30 % - in the main group ($P < 0,05$). The percentage of cells with toxic granulocyte in the controls increased from 3.50 ± 0.50 % to 25.60 ± 11.70 % while this parameter was virtually stable in the animals which obtained the SECPE (9.10 ± 3.90 %).

Table 1. Leukogram changes in the animals under the influence of the selenium-enriched chicory phytocomposite extracts prior to and after the acute and chronic irradiation exposure

Parameters	Measure unit	Groups of animals						
		Specific normal range	Initial level	After the irradiation exposure				Intact group (I)
				Acute irradiation		Prolonged irradiation		
				Control group (II)	Main group (IV)	Control group (III)	Main group (V)	
White blood cells	G per l	12.29±0.6	13.4±1.65	5.14±0.07*	8.39±1.28**	10.77±1.8	13.40±3.40	13.13±1.65
Metamyelocytes	G per l	0.03±0.01	0.01±0.01	0.02±0.01	0.05±0.02**	0.01±0.01	0.62±0.06*	0.01±0.01
Stab neutrophils	G per l	0.17±0.02	0.04±0.02	0.04±0.02	0.14±0.04**	0.39±0.04*	0.85±0.65	0.04±0.02
Segmented neutrophils	G per l	2.77±0.39	3.54±0.69	1.24±0.16*	1.57±0.48*	1.41±0.31*	2.04±0.73	3.26±0.92*
Eosinophils	G per l	0.34±0.03	0.02±0.02	0.04±0.02	0.18±0.07**	0.62±0. 34	1.06±0.44*	0.01±0.01
Monocytes	G per l	0.20±0.04	0.37±0.05	0.028±0.01*	0.14±0.03**	0.24±0.13	0.61±0.28	0.2±0.06
Lymphocytes	G per l	8.49±0.36	8.87±1.68	3.63±0.5*	6.13±1.33	8.69±1.59	9.59±3.22	9.79±1.16
Natural killers	G per l	0.03±0.026	0.02±0.02	0±0	0±0	0.037±0.035	0±0	0±0

* P<0.05 at the comparison with the initial and control groups' data; ** P<0.05 at the comparison of the main and control groups' parameters

A considerable and significant rise in the number of neutrophils with karyorrhexis after the acute irradiation was observed and made up 44.20 ± 13.80 % and 61.60 ± 17.10 % in the II and IV groups, respectively, while the intact controls' level was 3.50 ± 0.51 % ($P < 0.05$). Ridder's lymphocytes made up to 1.62 ± 1.19 % in the peripheral blood of the II group animals but were not found in the blood of the IV group rats. Bean-shaped lymphocytes made up 2.74 ± 0.94 % and 2.18 ± 0.64 %, respectively, in the controls and main group animals; this slightly exceeded their detection rate in the peripheral blood prior to the irradiation exposure (1.72 ± 0.46 %), but was still within the normal range (3.56 ± 0.49 %). "Dry-leaf" lymphocyte content grew considerably but statistically insignificantly up to 0.65 ± 0.61% after the acute irradiation in the controls and halved in the main group of animals obtained SECPE down to 0.30 ± 0.29 % what was practically equal to the specific norm – 0.23 ± 0.16 %.

The cell dissolution rate in the peripheral blood after the prolonged irradiation exposure in the III and V groups was much lower than after the acute irradiation and made up 18.20 ± 5.70 % and 19.60 ± 1.70 %, respectively. The number of cells with destroyed membranes in the III control group was equal to 52.18 ± 21.30 % what doubled that of the V group in which the rats obtained SECPE – 26.90 ± 25.40 %. The content of cells with toxic granularity and fragmented nucleus in the main group animals was 47.9 ± 18.48 % and 33.15 ± 22.9%, respectively, and tended to decrease when compared with the controls – 56.70 ± 4.20 % and 50.81 ± 15.40 %, respectively. It should be noted that there were considerable individual fluctuations of these toxic-inflammatory process parameters in the III group, and, especially, in the V group of rats. Moreover, there were no significant differences between these two groups and the other groups of animals, the baseline levels, and the specific norm ($P > 0.05$). At the peripheral blood examination for the lymphocyte pools in animals that had obtained SECPE, there were no Ridder's lymphocytes found neither after acute nor after prolonged irradiation exposure. The content of Ridder's lymphocytes in the controls after the prolonged irradiation exposure was 1.31 ± 0.99%, and that was somewhat lower than in the controls after acute irradiation. In contrast with the acute irradiation, the prolonged exposure in the main group compared with the controls, led to the tendency of "dry-leaf" lymphocyte content increase (0.91 ± 0.33% and 1.88 ± 0.90%, respectively), along with the decrease of bean-shaped lymphocyte count (5.9 ± 2.97% and 0.94 ± 0.4%, respectively).

The morphological examination of the main lymphocyte pools revealed the trend to decrease of the toxic-inflammatory process under the influence of the SECPE treatment course at both acute and prolonged irradiation exposure and demonstrated the high positive effect of the SECPE upon the lymphocytic pool at acute irradiation.

Figures 1 – 4 represent the auto sensitization levels under the SECPE action at the acute and prolonged irradiation exposure. As the data show, there was a considerable auto sensitization to all examined tissues at the acute irradiation. A high sensitization degree was detected in the controls (II group) to the following tissues: spleen – 0.38 ± 0.11 standard unit (SU), suprarenal gland – 0.42 ± 0.09 SU, testicle – 0.36 ± 0.09 SU, liver – 0.34 ± 0.06 SU, kidney – 0.38 ± 0.03 SU, heart – 0.27 ± 0.05 SU, lung – 0.34 ± 0.12 SU, and limbus – 0.32 ± 0.06 SU. Medium-degree sensitization was registered to the brainstem tissues – 0.2 ± 0.05 SU, and cerebral cortex – 0.23 ± 0.09 SU. Mild sensitization to cerebellar tissue 0.15 ± 0.11 SU was observed. In the main group of animals which obtained food supplementation with the SECPE no auto sensitization was detected: its levels did not exceed 0.07 ± 0.04 SU whereas the specific norm was up to 0.09 SU, and the initial levels ranged from 0.04 ± 0.01 SU to 0.08 ± 0.01 SU.

The controls of the III group after the prolonged irradiation exposure demonstrated high sensitization to the following tissues: spleen – 0.36 ± 0.08 SU, testicle – 0.39 ± 0.16 SU, liver – 0.52 ± 0.12 SU, heart – 0.33 ± 0.07 SU, lung – 0.37 ± 0.08 SU, and cerebral cortex – 0.56 ± 0.21

SU. Medium-degree sensitization to the suprarenal and renal tissues (0.22 ± 0.05 SU and 0.27 ± 0.16 SU, respectively) was found. The limbic and cerebellar tissues caused mild sensitization: 0.18 ± 0.08 SU and 0.19 ± 0.09 SU, respectively.

The animals of the V group which obtained SECPE, in contrast with the IV group rats which were exposed to the acute irradiation exposure, were sensitized to all the tested tissues without exception. Moreover, the sensitization level in the majority of the V group animals tended to increase when compared with the III group controls which were not administered SECPE. The V group revealed high sensitization level to the following tissues: spleen – 0.49 ± 0.17 SU, suprarenal gland – 0.42 ± 0.14 SU, testicle – 0.41 ± 0.19 SU, liver – 0.32 ± 0.21 SU, kidney – 0.50 ± 0.08 SU, heart – 0.47 ± 0.12 SU, lung – 0.42 ± 0.14 SU, brainstem – 0.48 ± 0.05 SU, and cerebral cortex – 0.45 ± 0.16 SU. Medium-degree sensitization to the cerebellar and limbic tissues was registered: 0.27 ± 0.10 SU and 0.20 ± 0.16 SU, respectively.

According to the data of non-specific resistance mechanisms investigation by the neutrophil bactericidal biochemical activity in the NBT test, in the II group animals after the acute irradiation exposure, the peripheral blood content of formazan-positive neutrophils and their activity index decreased considerably and significantly and made up 6.1 ± 0.06% and 14 ± 0.07 SU, respectively, while in the III group after the prolonged irradiation those parameters were 4.0 ± 0.04% and 0.15 ± 0.01 SU; the initial levels were equal to 17.51 ± 1.54% and 0.19 ± 0.014 SU, and the normal ranges were 17.96 ± 2.68% and 0.17 ± 0.25 SU, respectively (P> 0.05). In the IV and V main groups where the animals obtained SECPE, the formazan-positive neutrophil content and the neutrophil activity index were virtually similar to those of the specific norm and the baseline levels regardless of the irradiation exposure type and made up 15.0 ± 1.90% and 0.15 ± 0.01 SU in the IV group and 14.3 ± 4.48 % and 0.14 ± 0.07 SU in the V group, respectively. These witnesses to the substantial radioprotector effect of the SECPE with regard to the non-specific resistance mechanisms.

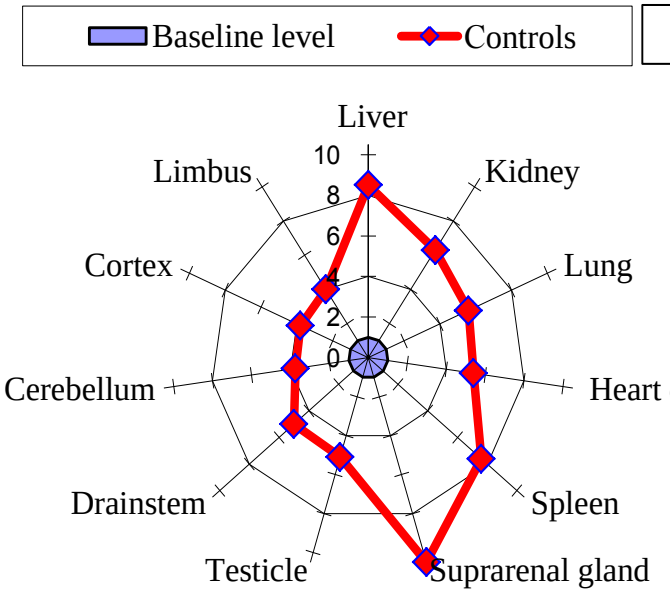

Fig. 1. Sensitization to the tissue antigens after the acute irradiation exposure in the controls (II group)

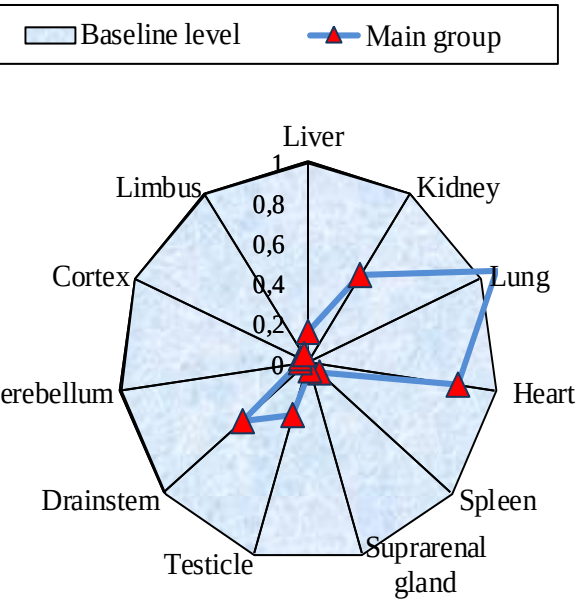

Fig. 2. Sensitization to the tissue antigens after the acute irradiation exposure in the main group animals which obtained food supplementation with SECPE (IV group)

The grooming activity of the controls after the acute irradiation exposure estimated by the washing, licking and scratching elementary grooming acts (EGA) made up 10.50 ± 1.90 EGA, 7.90 ± 3.00 EGA, and 6.50 ± 0.90 EGA, respectively; this was almost indistinguishable from the baseline (10.80 ± 0.05 EGA, 7.50 ± 0.35 EGA, and 7.10 ± 0.20 EGA, respectively), and the specific norm (11.8 ± 0.60 EGA, 7.50 ± 0.40 EGA, and 6.10 ± 0.30 EGA, respectively). The main group animals demonstrated a slight trend to the increased number of washing acts (12.50 ± 2.30 EGA) while the licking and scratching rates stayed the same (5.50 ± 1.20 EGA, and 9.60 ± 2.10 EGA, respectively).

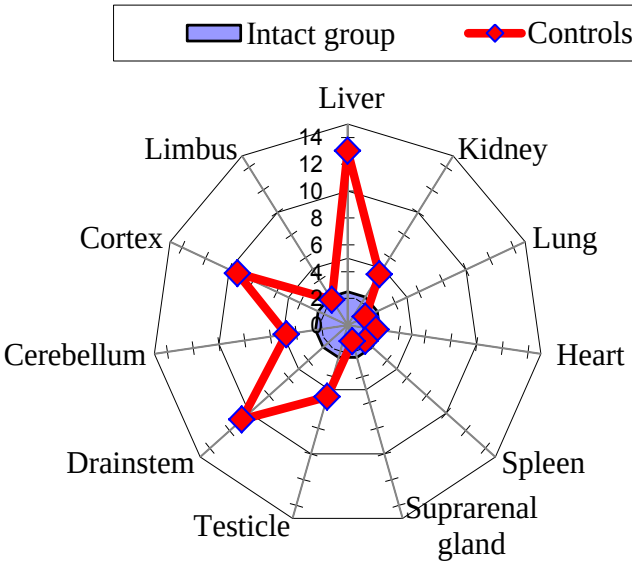

Fig. 3. Sensitization to the tissue antigens after the prolonged irradiation exposure in the controls (III group)

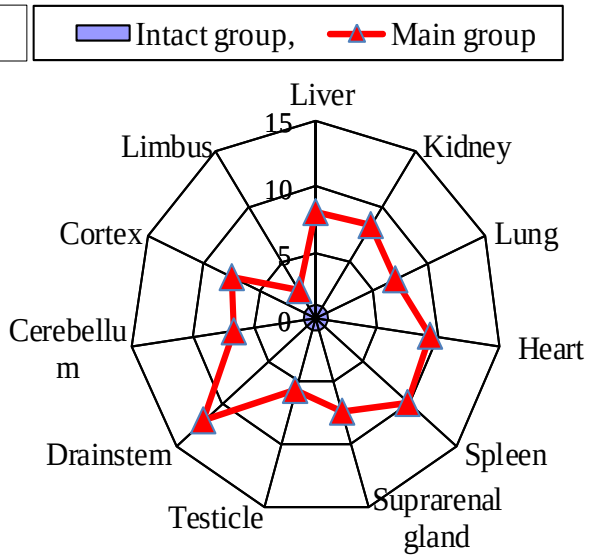

Fig. 4. Sensitization to the tissue antigens after the prolonged irradiation exposure in the main group animals which obtained food supplementation with SECPE (V group)

The controls after the prolonged irradiation course showed a significant increase of the washing activity (up to 17.20 ± 0.96 EGA) versus the baseline and specific norm ($P < 0.05$). In this group, the licking activity tended to increase (15.60 ± 5.00 EGA), whereas the scratching activity was unchanged (6.40 ± 4.00 EGA). The main group animals which were administered the SECPE, in contrast with the controls, demonstrated the stable washing activity without an increase (10.00 ± 2.11 EGA) what was indistinguishable from the baseline and specific norm levels (10.80±0.50 EGA, and 11.80±0.60 EGA, respectively). The number of licking and scratching acts in the V group rose significantly and made up 24.8 ± 1.34 EGA and 17.2 ± 3.24 EGA, respectively.

4. Conclusions

The study demonstrates the radioprotective effect of the SECPE at both acute and prolonged irradiation exposure. This effect is based on the high biological activity of the SECPE which stimulates, regulates, and restores the psychoneuroimmune system and the non-specific resistance mechanisms. In turn, the adaptation capability is extended and the body tolerance

including that towards ionizing irradiation is increased. In our opinion, the SECPE radioprotective effect is most expressed at acute irradiation exposure.

Based on the SECPE, we have elaborated a dietary food supplement with biological activity – "Selenium-S" [20], which is promising both being used *per se* and for functional products development with the purpose of psychoneuroimmune regulation improvement including cases of radiation damage and requires further investigation of use peculiarities.

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ИССЛЕДОВАНИЕ СЕЛЕНСОДЕРЖАЩИХ ФИТОКОМПОЗИТОВ ЦИКОРИЯ КАК РАДИОПРОТЕКТОРА ПРИ ОСТРОМ И ХРОНИЧЕСКОМ ОБЛУЧЕНИИ

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Резюме: Радиопротекторные свойства селенсодержащих фитокомпозитов цикория (ССФКЦ) были изучены в серии экспериментов на животных. Показано, что селен является основным материалом для пищевых добавок и продуктов функционального питания для защиты от острого и хроническго облучения. Исследование демонстрирует радиопротекторный эффект SECPE как при остром, так и при разных режимах облучении. Этот эффект основан на высокой биологической активности ССФКЦ, которая стимулирует, регулирует и восстанавливает психонейроиммунную систему и механизмы неспецифической резистентности. По нашему мнению, радиозащитный эффект ССФКЦ наиболее выражен при остром облучении. На основе ССФКЦ мы разработали биологически активную добавку к пище с биологической активностью - «Селен-С», которая является перспективной как для использования как таковая, так и для разработки продуктов с целью улучшения психонейроиммунной регуляции, включая случаи радиационного повреждения, и требует дальнейшего исследование особенностей использования.

Ключевые слова: обогащенный селеном цикорий, фитокомпозитный экстракт, острая и пролонгированная радиация, радиопротектор.

KƏSKİN VƏ XRONİK ŞÜALANMAYA QARŞI RADİOPROTEKTOR KİMİ SELENLİ HİNDİBA FİTOKOMPOSİTLƏRİN TƏDQİQİ

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Xülasə: Seleniumly hindiba fitokompositlərin (SHFK) radioprotektor xüsusiyyətləri bir sıra heyvanlarla təcrübələrdə tədqiq edilmişdir. Selenyum, qida əlavələri və funksional qidalar üçün əsas materialdır ki, kəskin və uzun müddətli şualanma təsirinə qarşı qorunma bacarağı olduğu göstərilmişdir. Tədgigatlar göstərir ki, SHFK-in radioprotektiv təsiri mövcuddur, həm də kəskin və uzun muddətli radiasiya rejiminə də aiddir. Bu təsir, psixoneuroimmun sistemini və qeyri-spesifik müqavimət mexanizmlərini stimullaşdırır, tənzimləyir və bərpa edən SHFK-ların yüksək radioprotektorluk bioloji fəaliyyətinə əsaslanır. Bizim fikrimizcə, SHFK-nin radiasiya qoruyucu təsiri kəskin şualanma zamanı ən bariz meydana çıxır. SHFK-i əsas götürərək, bioloji aktiv madda hazirlanib ki, Selen-C ilə birlikdə qida üçün də istifadə etmək olar və həm də şualanma zədələnmə hadisələri də daxil olmaqla, psixoneuroimmun tənzimlənməsini optimal etdirmək üçün bu məhsulların perspektivli göstərilir və gələcək tədqiqatlarda onların istifadə edilməsi öyrəniləcək.

Açar sözlər: seleniumla zənginləşdirilmiş hindiba, fito-kompozit ekstraktı, kəskin və uzun müddətli şualanma, radioprotektor.