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Application of the Se NPs-Chitosan molecular complex for the correction of selenium deficiency in rats model

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ABSTRACT

Selenium is an integral component of vital biologically active compounds of the human body. Currently, the population of many countries is characterized by selenium deficiency. In this regard, many preparations of inorganic and organic forms of selenium have been developed. Nevertheless, it is evident that the most effective solution to the problem is to enrich the diet with bioavailable forms of selenium. Thus, this work aimed to synthesize and study the antioxidant and immunomodulatory effects of the molecular complex of selenium nanoparticles (Se NPs) and chitosan in laboratory rats with induced hyposelenosis. During the experiment with animals, we found that as a result of 70-day consumption of food with a low selenium content, rats develop an alimentary selenium deficiency state, as evidenced by a significant decrease in the content of this trace element in control group rats to 48.2 ±6.71 μg/kg versus 149.3 ±21.63 μg/kg in intact animals. Course, administration of the molecular complex Se NPs- Chitosan to rats of the experimental group, contributed to the replenishment of selenium deficiency: its concentration in the blood of animals was 96.6 ±3.57 μg/kg. Thus, in animals of the control group, there was a decrease in the total number of lymphocytes by 2.7 times, T-lymphocytes – by 1.8 times, and B-lymphocytes – by 2.3 times compared with similar data in intact animals. In the context of hyposelenosis, it is worth mentioning that there was a slight increase in the content of T-helper cells and cytotoxic T-lymphocytes. The synthesized Se NPs – Chitosan complex administration during hyposelenosis demonstrated a notable immunomodulatory effect by restoring the body's immune response indicators. Thus, the total number of lymphocytes increased by 3 times, T-lymphocytes – by 1.9 times, and B-lymphocytes – by 2 times. The number of T-helper cells and cytotoxic T-lymphocytes increased by 1.9 times compared to the group of intact animals and 1.6 times compared to selenium-deficient rats. Thus, the course introduction of the molecular complex Se NPs – Chitosan against the background of selenium deficiency was accompanied by inhibition of free radical oxidation processes, activation of the antioxidant system and restoration of the immune status of the organism of laboratory animals.

Keywords: selenium, polysacharides, selenium deficiency, immunity

INTRODUCTION

The organism's most pronounced dependence on biogeochemical factors manifests in the form of endemic diseases caused by a sharp deficiency, excess or imbalance of trace elements in the biogeochemical food chain **[1]**. One of the essential trace elements is selenium. It is an integral component of more than 30 vital biologically active compounds of the human body **[2]**. As part of the antioxidant enzyme glutathione peroxidase, selenium protects cells from excess oxygen, peroxides and free radicals **[3]**, **[4]**. Selenium stimulates the conversion of methionine into cysteine and the synthesis of glutathione **[5]**. The selenium protein complex catalyzes the biosynthesis of thyroid hormones **[6]**. About 75 different pathologies are associated with the deficiency of this trace element. People with hyposelenosis have a low life expectancy due to premature ageing **[7]**. Selenium is

necessary for the normal functioning of the immune system, both cellular and humoral: it stimulates the function of natural killers **[8]**; increases the production of interleukin-1 and interleukin-2 **[9]**; suppresses immediate-type hypersensitivity and delayed-type hypersensitivity **[10]**; modulates the phagocytic function of polymorphonuclear leukocytes **[11]**; potentiates the function of natural killers and antibody genesis **[12]**; has anti-apoptogenic and radioprotective effects **[13]**; blocks the transcription of viruses, including the AIDS virus **[14]**. Selenium has a powerful immunomodulatory activity **[15]**.

Selenium enters the body with food and water. Depending on the type of soil and the underlying rocks, a different amount of it is assimilated by plants and gets into human and animal food **[16]**. Regions with a selenium content in the soil below 50 µg/kg are endemic **[17]**. The low content of trace element in soils is associated with its lack in the underlying soils; the presence of a layer of permafrost prevents its leaching from deep layers into surface ones; intensification of agricultural production **[18]**. An adequate dose of selenium, depending on the region of residence, ranges from 50 to 200 µg/day and is at least 70 µg for adult men and 55 µg for adult women (at least 1 µcg/kg/day) **[19]**. One of the most important advantages of biologically active additives (dietary supplements) with organic selenium compounds is, in addition to low toxicity, their wide possibilities for accumulation and deposition in the body. When an excess of selenomethionine and selenocysteine enter the body, they are easily incorporated into protein molecules instead of methionine and cysteine **[20]**. The capacity of the "protein depot" in the body is quite large. This is due to the low toxicity of selenomethionine compared to sodium selenite **[21]**. Recently, complex dietary supplements based on inorganic and organic selenium compounds have been developed and proposed for practical use **[22]**. At the same time, the list of selenium-containing medicines is not so large, and some of them have several disadvantages, such as toxicity, rapid elimination from the body, and some others **[23]**.

Moreover, a more effective solution to the problem of selenium deficiency is associated with the enrichment of food products in the daily diet instead of numerous offers of selenium-containing medicines. In this regard, the current direction of the modern food industry is the development of new selenium-containing complexes, the most effective and safe for use in food products. Thus, this work aimed to synthesize and study the antioxidant and immunomodulatory effects of the molecular complex of selenium nanoparticles (Se NPs) and chitosan in laboratory rats with induced hyposelenosis.

Scientific Hypothesis

The use of the bioavailable molecular complex Se NPs-Chitosan in the diet is an effective solution to the problem of selen deficiency. The supplementation of feed with molecular complex Se NPs-Chitosan will increase Se content in rats' blood. Also, we are expecting that the course introduction of the molecular complex Se NPs-Chitosan against the background of selenium deficiency will cause inhibition of free radical oxidation processes, activation of the antioxidant system and restoration of the immune status of the organism of rats.

MATERIAL AND METHODOLOGY

Samples

Molecular complex Se NPs-Chitosan.

Chemicals

We used reagents of recognized analytical purity and distilled water. The work used the following chemicals: Ethanol, Sodium hydroxide, Sodium Selenite, Ascorbic acid, and Chitosan. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality.

Animals and Biological Material

Experimental work was carried out on 50 white male rats weighing 150-160 g in standard vivarium conditions.

Instruments

Magnetic Stirrer IKA I-MAG (ChimMed, Russia), pipet dispenser Vitlab micropipette (Vitlab, Moscow, Russia), biochemical blood analyzer Olympus AU 400 (Olympus Europa SE & Co. KG, Hamburg, Germany), hematological analyzer MEK 7222 (NihonKohden, Tokio, Japan), flow cytofluorimeter Cytomics FC 500 (Beckman Coulter, New York, USA), the device for the total determination of antioxidants Tsvet Yauza-AA-01 (ChemAutomatika, Moscow, Russia), liquid chromatograph Shimadzu 20-AD (Shimadzy, Tokio, Japan), X-ray diffractometer Shimadzu XRD 7000 (Shimadzy, Tokio, Japan), spectrophotometer Shimadzu UV-2600 (Shimadzy, Tokio, Japan), laser analyzer Shimadzu Sald 2300 (Shimadzy, Tokio, Japan), scanning electron mycroscope Sigma Ziess (Carl Zeiss QEC GmbH, Koln, Germany).

Laboratory Methods

Sodium selenite and ascorbic acid were used to obtain Se NPs in solution. Se NPs were stabilised using chitosan with a degree of deacetylation of about 75%. Transmission electron microscopy was performed using a

scanning electron microscope Sigma Zeiss (Carl Zeiss QEC GmbH, Cologne, Germany). X-ray diffraction analysis was performed using X-ray diffractometer Shimadzu XRD 7000 (Shimazu, Tokyo, Japan). The size distribution was studied using laser analyzer Shimazu Salt 2300 (Shimaji, Tokyo, Japan). Optical properties of the synthesized molecular complex were sudied with spectrophotometer Shimadzu UV-2600 (Shimaji, Tokyo, Japan).

The fluorimetric method determined the selenium content in the blood of experimental animals **[24]**. Indicators of the immune status of the body: the total number of lymphocytes, T-lymphocytes (TL), T-helper (TH) cells $CD^{3+}CD^{4+}$, cytotoxic T-lymphocytes (CTL) $CD^{3+}CD^{8+}$, B-lymphocytes (BL), natural killers (NK), were determined by the method of flow cytofluorimetry **[25]**. To assess the activation of autoimmune processes, the ratio of TH/CTL was calculated **[26]**. The intensity of free radical oxidation processes in blood plasma and erythrocytes was determined by the accumulation of malondialdehyde (MDA) **[27]**. Overall antioxidant activity was determined as the main indicator of antioxidant protection **[28]**.

Description of the Experiment

Sample preparation: The Se NPs-Chitosan molecular complex was prepared as follows: ascorbic acid (0.35 g/l) and sodium selenite (0.15 g/l) were added to 0.25 g/l chitosan solution. The resulting solution was thoroughly mixed on a magnetic stirrer IKA I-MAG (ChimMed, Russia) for 30 minutes. As a result of the redox reaction, a red colloidal solution was formed.

Number of samples analyzed: 3.

Number of repeated analyses: 3.

Number of experiment replication: 1.

Design of the experiment: At the first stage, the molecular complex Se NPs-Chitosan was synthesized. Various research methods were used to characterize the synthesized molecular complex. The obtained samples' structure, shape, morphology, and size were investigated. Hyposelenosis in laboratory animals was modelled by alimentary selenium deficiency, for which the animals were kept on a diet with a low selenium content (14 µg/kg) for 70 days. Animals with hyposelenosis were intragastrically administrated with Se NPs-Chitosan complex at a dose of 0.5 µg/kg in a volume of 10 ml/kg once a day for 10 days. Animals of the control group received an equivalent volume of distilled water according to a similar scheme. For the purity of the experiment, a group of intact rats with the usual daily diet, which did not cause hyposelenosis, was also used in the work.

Statistical Analysis

Statistical processing of the results was performed with elements of nonparametric statistics using the Statistica 12.0 software package (StatSoft, USA) using the Student's T-test (p <0.05).

RESULTS AND DISCUSSION

Figure 1 shows the characteristics of the resulting NPs. The NPs consist of crystalline selenium, have a spherical shape, and have a diameter of 30-40 nm, typical for Se NPs stabilized with polysaccharides **[29]**. The electromagnetic absorption spectrum in the UV/visible region peaks at 400 nm, similar to the results obtained in other works **[30]**, **[31]**. According to previous studies, the effect of the selenium: polysaccharide mass ratio was determined by the saturation region of the adsorption capacity, which affects the formation process and morphological characteristics of nanostructures **[29]**, **[32]**. According to UV spectroscopy, viscometry and pH-metre, this region corresponds to a range of mass ratios (v) from 0.02 to 0.08 **[33]**. In this work, a selenium nanocomposite with a mass ratio of v = 0.04 was used, a selenium nanocomposite with a mass ratio of v = 0.04 was used The X-ray diffraction pattern showed the presence of peaks characteristic of polycrystalline selenium **[34]**. Thus, we have confirmed that nanoscale selenium has been synthesized. During the 10 days of the experiment, the particles did not aggregate, which confirms the stability of the Se NPs-Chitosan molecular complex.

Similar Se NPs-Chitosan complexes have been synthesized and studied by other researchers. Song et al. used TEM to study the cellular uptake and intracellular distribution of Se NPs modified with chitosan in HepG2 cells **[35]**. Se NPs have been localized in intracellular structures such as endosomes and lysosomes. El-Megharbel et al. demonstrated that the novel Se NPs-Chitosan complexes had a potent effect against Diclo-Na-induced testicular toxicity and hormonal disturbance in male rats, particularly high oxidative stress, thus protecting against testicular necrosis and dysfunction **[36]**. Abozaid et al. demonstrated the therapeutic effect of Se NPs-Chitosan against experimentally induced diabetes mellitus in adult male rats **[37]**. This effect is afforded by the antioxidant, hypoglycemic, and hypolipidemic properties of Se NPs-Chitosan, resulting from using selenium and chitosan. All these findings point out the relevance and importance of the study and characterization of synthesized Se NPs-Chitosan complex.

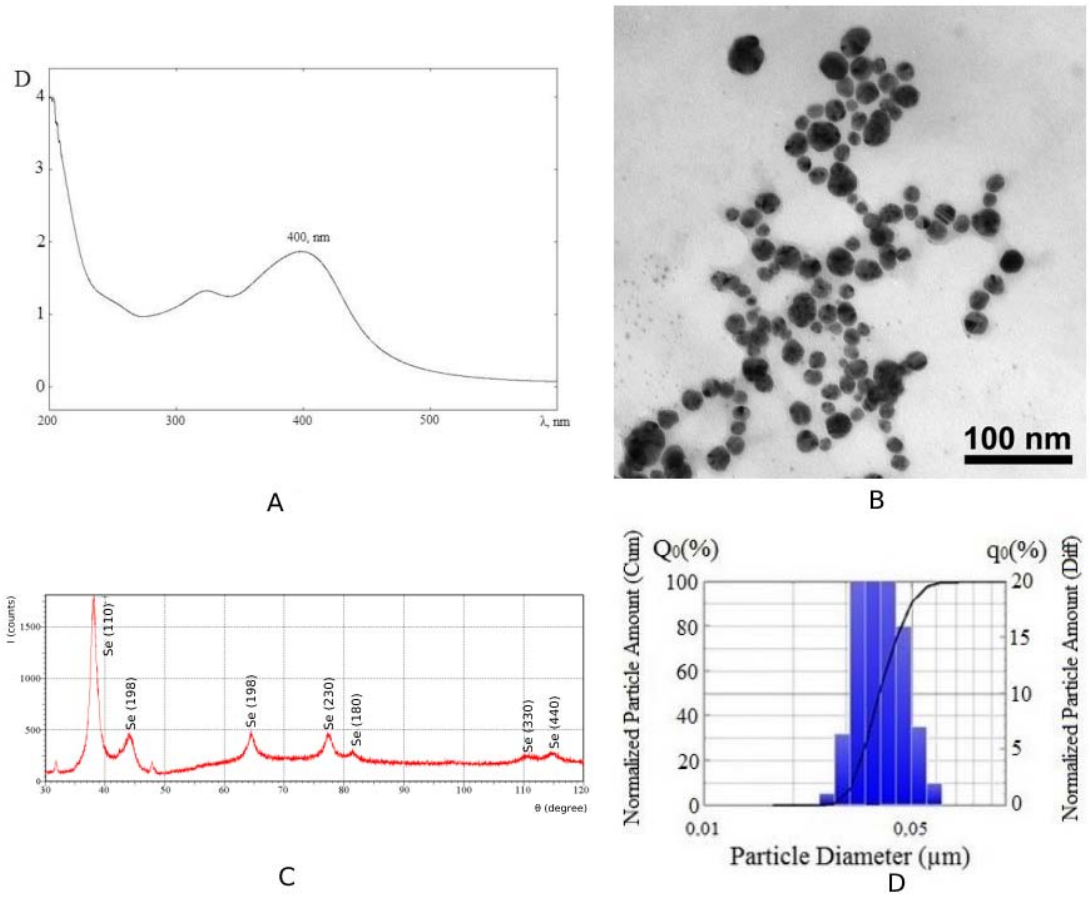

Figure 1 Characteristics of obtained Se NPs stabilized with Chitosan. Note: UV/visible absorption spectrum (A), transmission electron microscopy (B), X-ray pattern (C), size distribution of nanoparticles (D).

During the experiment with animals, we found that as a result of a 70-day intake of food with a low selenium content, rats develop a nutritional selenium-deficient state, as evidenced by a significant decrease in the content of this trace element in rats of the control group to 49.2 ±6.2 μg/kg versus 129.8 ±22.3 μg/kg in intact animals. The course administration of the molecular complex Se NPs-Chitosan to the rats of the experimental group contributed to the replenishment of the deficiency of selenium: its concentration in the blood of the animals was 99.1 ±5.6 μg/kg (differences compared with the data of the rats of the control group are significant, $p < 0.05$). Our results showed that, against the background of selenium deficiency, animals develop oxidative stress, as evidenced by a significant increase in the concentration of MDA in the blood serum and erythrocytes of rats in the control group, as well as a decrease in the total antioxidant activity (Table 1). Other works also confirm this **[38]**, **[39]**.

Table 1 Effect of the molecular complex Se NPs-Chitosan on the parameters of free radical oxidation processes in experimental selenium deficiency.

Index	Group		
	Intact group $n = 10$	**Control group** $n = 10$	**Experimental group** $n = 10$
MDA in blood serum, μmol/mg lipids	3.6 ±0.1	9.7 ±0.6	3.9 ±0.4
MDA in erythrocytes, μmol/mg lipids	42.3 ±1.2	113.8 ±5.5	45.7 ±1.3
Total antioxidant activity, %	4.5 ±0.1	2.1 ±0.1	4.4 ±0.1

Note: Significance of differences ($p < 0.05$) was observed between all groups.

A two-fold average reduction in the concentration of MDA in the blood serum and erythrocytes of the animals in the experimental group, as well as an increase in the total antioxidant activity, show that the course administration of the test agents against the background of selenium deficiency had an antioxidant effect. It should

be highlighted that following the addition of the synthetic compound Se NPs-Chitosan, the levels of MDA and total antioxidant activity were reduced to those of intact laboratory animals, and the levels of these antioxidants were increased.

Moreover, hyposelenosis has been shown to cause immunodeficiency in experimental animals, as shown by a sharp decline in the parameters of the humoral connection to immunity (Table 2) **[40]**. Thus, in animals of the control group, a decrease in the total number of lymphocytes was observed to 2.7 times, TL – by 1.8 times, BL – by 2.3 times compared with similar data in intact animals. It should be noted that against the background of hyposelenosis, the content of TC and CTL slightly increased, but the ratio of TC and CTL practically did not change. The course introduction of the synthesized complex Se NPs-Chitosan against the background of hyposelenosis had a significant immunomodulatory effect, restoring the parameters of the body's immune response. Thus, the total number of lymphocytes increased 3 times, TL – 1.9 times, BL – 2 times. The amount of TC and CTL in such rats only tended to increase: their number increased by 1.9 times compared with the group of intact animals and by 1.6 times compared with selenium-deficient rats. Similar results have been previously reported by Bai et al. **[41]**. At the same time, the content of NK decreased (differences are statistically significant, $p < 0.05$) in the experimental group. Obviously, this phenomenon is explained by the fact that these cells perform their function much earlier than CTL and are the "first line" of the body's defense. Therefore, a decrease in their number is observed against the background of activation of the adaptive link of immunity **[42]**, **[43]**. The data obtained indicate that using the Se NPs-Chitosan molecular complex not only restores the parameters of the immune response, but also causes a pronounced activation of the adaptive link of immunity, which is confirmed in other works **[44]**, **[45]**, **[46]**. The immunity of rats with hyposelenosis in the experimental group was restored to the physiological norm.

Table 2 Influence of the molecular complex Se NPs-Chitosan on indicators of immunity in the selenium-deficient state in animals.

Index	Group		
	Intact group ***n*** **= 10**	**Control group** ***n*** **= 10**	**Experimental group** ***n*** **= 10**
Total number of lymphocytes, %	60.7 ±4.2	22.5 ±3.4	67.7 ±5.2
TL, %	75.5 ±3.6	41.6 ±4.5	79.6 ±6.1
TH ($CD^{3+}CD^{4+}$),%	51.2 ±3.3	56.3 ±5.1	49.9 ±2.8
CTL ($CD^{3+}CD^{8+}$), %	37.2 ±2.1	42.1 ±3.6	70.1 ±6.2
TH/CTL	1.37 ±0.1	1.4 ±0.1	0.7 ±0.1
BL, %	70.1 ±3.2	31.4 ±2.4	62.7 ±5.3
NK, %	12.8 ±0.3	13.9 ±0.7	9.6 ±0.6

Note: TL – T-lymphocytes, TH – T-helpers, CTL – cytotoxic T – lymphocytes, BL – B-lymphocytes, NK – natural killers. The significance of differences ($p < 0.05$) was observed between all groups.

Thus, the course administration of the Se NPs-Chitosan molecular complex against the background of a selenium-deficient state was accompanied by inhibition of free radical oxidation processes, activation of the antioxidant system, and restoration of the immune status of the body of laboratory animals. It is known that selenium is an indispensable component of the immune control system **[47]**. However, the synthesized molecular complex also contained chitosan, an immunomodulator **[48]**, **[49]**, **[50]**. In particular, the antioxidant mechanism of action of the synthesized molecular complex can also be based on the ability to protect capillary walls from the damaging effects of free radicals by neutralizing reactive oxygen species and terminating free radical chain reactions **[51]**, **[52]**.

With selenium deficiency, a lack of deiodinases of various types is formed, the formation of TL decreases, leading to stimulation of the hypothalamic-pituitary axis by the negative feedback system and an increase in the synthesis of thyroid-stimulating hormone (TSH) **[6]**. TSH stimulates the production of thyroid hormones and increases the activity of deiodinases, restoring the level of thyroid hormones. But at the same time, it stimulates the formation of hydrogen peroxide, for the inactivation of which, again, selenoprotein – glutathione peroxidase is required, the activity of which is reduced in conditions of selenium deficiency **[53]**. Hydrogen peroxide accumulates in the thyroid gland, which leads to damage to thyrocytes and the development of fibrosis **[54]**. Increased formation of hydrogen peroxide in the thyrocyte is observed in all cases of excessive thyroid stimulation by TSH, for example, in patients with autoimmune thyroiditis and subclinical hypothyroidism. As a result, there is damage to thyrocytes, the progression of hypothyroidism and the development of fibrosis. In such a situation, selenoproteins, having antioxidant activity, can prevent or at least slow down the destruction of thyrocytes and decrease their functional activity **[55]**.

Selenium-containing enzymes (iodothyronine deiodinase, glutathione peroxidase, and thioredoxin reductase), in addition to influencing thyroid metabolism, also play a significant role in organ-specific immune reactions **[11]**. According to several scientific papers, selenium in chronic inflammatory lesions of the thyroid gland protects follicles from oxidative stress and infiltration by autoreactive cells, reducing the production of pro-inflammatory cytokines **[56]**, **[57]**, **[58]**. Moghaddam et al. showed that selenium deficiency and, accordingly, a decrease or absence of glutathione peroxidase activity contributes to oxidative damage, thyroid damage and the development of fibrosis **[59]**. It can be assumed that even with moderate selenium deficiency, this mechanism is an important environmental factor initiating or supporting autoimmune thyroiditis **[60]**. Glutathione peroxidase can reduce the concentration of hydrogen peroxide and hydroperoxides, thereby reducing the spread of free radicals and reactive oxygen species. A decrease in the concentration of lipid hydroperoxides and phospholipids reduces the production of inflammatory cytokines **[61]**. A possible decrease in the immunomodulatory effects of glutathione peroxidase and thioredoxin reductase in selen deficient conditions switches the cytokine pattern towards a Th-2-dependent immune response, which leads to an intensification of inflammatory reactions in the body against the background of autoimmune processes or infections.

Thus, selenium-dependent enzymes give antioxidant and anti-inflammatory effects. This is because selenoproteins reduce lipid and phospholipid hydroperoxides, reducing the amount of free radicals and reactive oxygen species. A decrease in the concentration of hydroperoxides in tissues inhibits the formation of inflammatory prostaglandins and leukotrienes **[62]**. This mechanism may contribute to a decrease in inflammatory activity in the organ-specific autoimmune response and may serve as an explanation for the data obtained on a decrease in the content of lymphocytes **[63]**. Probably, a significant decrease in the concentration of natural killers in animals of the experimental group was achieved due to the indicated mechanism of selenium exposure. Our studies have shown that even with a slight deficiency of selenium, additional intake of this trace element has a clinically significant effect on the anti-inflammatory activity of the body.

The molecular complex Se NPs-Chitosan has a wide range of potential applications in specialized food products for therapeutic and prophylactic appointments. For example, it can be used as an alternative to the delivery of bioactive peptides with the potential as an emulsion stabilizer for food applications **[64]**, as a composite for active food packaging **[65]**, **[66]**, for extension of shelf life of dairy products **[67]** and minced meat **[68]**. Chen et al. reported that Se NPs-Chitosan complex has anticancer, anti-diabetic, antibacterial, and hepatoprotective activities and can improve the nutraceutical value of animals and crops for human consumption **[69]**. Golmohammadi et al. also confirmed that the nano complex Se NPs-Chitosan might be a development for treating diabetic wound infection at mild stage **[70]**. All this determines future directions in studying and applying the synthesized molecular complex Se NPs-Chitosan.

CONCLUSION

For the experiment, we synthesized the Se NPs-Chitosan molecular complex. It is established that the nanoparticles consisted of crystalline selenium, and had a spherical shape with a diameter of 30-40 nm. The electromagnetic absorption spectrum in the UV/visible region showed a peak of 400 nm. The X-ray showed the presence of peaks characteristic of polycrystalline selenium. Thus, we confirmed that we synthesized nanoscale selenium. During 10 days of the experiment, the particles were not aggregated, which confirms the stability of the Se NPs-Chitosan molecular complex. During the experiment with animals, we found that as a result of 70-day consumption of food with a low selenium content, rats developed an alimentary selenium deficiency state, as evidenced by a significant decrease in the content of selenium in the control group rats to 49.2 ±6.2 μg/kg versus 129.8 ±22.3 μg/kg in intact animals. Course administration of the molecular complex Se NPs-Chitosan to rats of the experimental group contributed to the replenishment of selenium deficiency: its concentration in the blood of animals was 99.1 ±5.6 μg/kg. Thus, in animals of the control group, there was a decrease in the total number of lymphocytes by 2.7 times, T-lymphocytes – by 1.8 times, and B-lymphocytes – by 2.3 times compared with similar data in intact animals. It should be noted that against the background of hyposelenosis, the content of T-helper cells and cytotoxic T-lymphocytes increased slightly. The course administration of the synthesized Se NPs-Chitosan complex against the background of hyposelenosis had a significant immunomodulatory effect, restoring the indicators of the body's immune response. Thus, the total number of lymphocytes increased by 3 times, T-lymphocytes – by 1.9 times, and B-lymphocytes – by 2 times. The number of T-helper cells and cytotoxic T-lymphocytes increased by 1.9 times compared to the group of intact animals and 1.6 times compared to selenium-deficient rats. Thus, the course introduction of the molecular complex Se NPs-Chitosan against the background of selenium deficiency was accompanied by inhibition of free radical oxidation processes, activation of the antioxidant system and restoration of the immune status of the organism of laboratory animals. The obtained results provide a basis for further exploration of the Se NPs-Chitosan molecular complex in developing specialized food products for therapeutic and prophylactic applications.

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