Original Article

Cytomorphological assessment of the effect of selenium nanoparticles on SPEV-2 oncovirus cells

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ABSTRACT

The activity of selenium nanoparticles on a SPEV-2 culture using propidium iodide (PI) was studied. Amon the range of studied concentrations, 32 and 320 µg/mL had cytostatic effects on SPEV-2 cells, while 3.2 and 32 µg/mL had cytotoxic effects. During the day, selenium nanoparticles caused apoptosis in cells at concentrations of 32 and 320 µg/mL. The superimposition of photographs under different microscopic modes of light scattering and fluorescence registration allowed us to describe cytomorphological indicators marking the process of cell death under the action of an extract inducing DNA damage in cells under *in vitro* conditions. The appearance of dark formations in the cytoplasm, fuzzy contours of the cells themselves, and their acquisition of a stellate shape or preservation of a rounded, but with the fragmentation of the cell membrane and leakage of contents, as well as formation of conglomerates by dead cells, were observed. A decrease in the intensity of the PI glow indicates the decay of the DNA of the nucleus. The index of the number of dead cells can serve as an index of cytotoxicity.

Keywords: Propidium iodide, Apoptosis, Proliferation, Nanotechnology

Introduction

Selenium is a trace element, by its chemical nature belonging to a group of non-metals. When ingested in an inactive form, it undergoes several metabolic transformations and, together with proteins, forms selenoproteins, which play an important role in maintaining homeostasis [1, 2]. At the same time, selenium nanoparticles are a promising supplier of biologically active substances to animals and humans, since selenium is indispensable for the vital activity of mammals [3]. Many human diseases are associated with a deficiency of this trace element, including a direct link between the trace element and hepatitis [4, 5], a link between selenium and diabetes [6, 7], as well as several cardiovascular diseases [8, 9]. The literature provides data on the association of this trace element with Alzheimer's disease [10], cancer [11], asthma [12], and cerebrovascular insufficiency [13]. It is worth noting the relationship between selenium and the ability to reduce the severity of heavy metal poisoning [14]. Notably, this effect is noted not due to the formation and binding of heavy metals into any complex, but the strengthening of the protective functions of the body by selenium, which is confirmed by low concentrations of selenium-containing additives having a positive impact [15]. Special attention is paid to the possibility of a positive effect of this trace element in diseases of the thyroid gland [16].

Signs specific to selenium deficiency in the absence of vitamin E include pancreatic degeneration, as well as poor growth,

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reproductive failure, vascular changes, and cataracts [17]. Generally, selenium deficiency in nature leads to many different diseases due to decreased immunity. With selenium deficiency, reproductive function decreases, and the organism's growth slows down [18]. With a lack of selenium in the body, lipid, carbohydrate and fat metabolism are disrupted [19]. Selenium deficiency also can lead to the emergence and reproduction of atypical cells and oncology [20]. At the same time, if a sufficient amount of selenium is injected into the body, then the enzymes manage to calculate and destroy this atypical cell [21]. Thus, selenium nanoparticles not only prevent the formation of these cells but also destroy those that have already formed [22, 23]. Selenium has a positive effect on all tumor processes, including blood diseases and various benign neoplasms [24, 25].

It was revealed that selenium nanoparticles have high bioavailability while having lower toxicity than other inorganic analogs [26, 27]. The unique features of nanoparticles, such as small size, high surface area, surface charge, solubility, and versatility, have successfully proven their ability to be used as carriers of therapeutic molecules [28]. Nanoparticles solve many biopharmaceutical and pharmacokinetic problems associated with many drugs in various classes of diseases [29]. They increase the therapeutic effectiveness of ionized drugs, as well as improve the penetration of water-soluble compounds, proteins, peptides, vaccines, miRNA, DNA, and other biological therapeutic drugs [30, 31]. The surface modification of nanoparticles with target ligands makes the drug delivery system much more versatile and can selectively deliver to the target object [32].

Thus, the use of antitumor agents based on selenium nanoparticles can achieve a pronounced therapeutic effect with minimal toxic effects [33, 34]. Recent studies showed that selenium nanoparticles, along with low toxicity, have antioxidant, antitumor, antimicrobial, and immunomodulatory properties [35].

Therefore, this study aimed to reveal the dynamics of cytomorphological changes in the SPEV-2 culture under the action of different concentrations of selenium nanoparticles. SPEV-2 culture carries oncoviruses "a" and "c". Therefore, it was used as a model of mammalian tumor cells *in vitro* to determine the cytotoxic and cytostatic effects of selenium nanoparticles.

Materials and Methods

For the experiment, selenium nanoparticles were synthesized at the Institute of General Physics (Moscow, Russia). The characterization of selenium nanoparticles using scanning electron microscopy and transmission electron microscopy showed the spherical shape of nanoparticles with a size of 30-90 nm, which determines the high activity of the obtained nanoparticles [36].

The object of the research was the SPEV-2 culture obtained from the cryobank of the cell culture collection (Ekaterinburg, Russia). Cultivation was carried out in RPMI 4 medium (10% embryonic serum, gentamicin, ampicillin, amphotericin) in 6 well plates (one control and five experimental wells) [37]. The control was cells in an environment without selenium nanoparticles that grew during the day. Selenium nanoparticles were diluted in a nutrient medium with a 10-fold decrease in concentration. Then solution of selenium nanoparticles was introduced into five wells: 3.2 μg/mL; 32 μg/mL; 320 μg/mL; 3.2 mg/mL and 32 mg/mL. The cells were cultured in a CO_2 incubator at 37 °C for 24 h. Propidium iodide (PI) was used as a dye, which penetrates cells due to the destruction of their membrane and binds to nuclear DNA [38]. To visualize the cells, a combination of several microscopic modes of light scattering and fluorescence detection on a Leica DM 2500 microscope, a Leica DFC 420C digital video camera with Leica Application Suite V 3.1 software was used [39].

The following average values were used to analyze cells in control and experimental cultures: the total cell number (TCN) in the field of vision, indicating proliferative activity; dead cell number (DCN) in the field of vision stained orange with propidium; and the DCN/TCN ratio in the field of vision multiplied by 100 %, reflecting the cytotoxic effect [40]. Cytomorphological signs were also noted during microscopy in phase contrast: the shape of cells, the appearance of characteristic intracellular granulation, the state of the cell membrane, etc [41]. The appearance of characteristic intracellular granulation was considered as evidence of the development of necrobiotic processes in the cell, their separation from the substrate, and subsequent death [42]. The decay of nuclear DNA into fragments of unequal size (under fluorescent conditions) was regarded as a sign of apoptosis [43].

Statistical data processing was performed using "Satistica 12.0" software. The significance of differences in the parametric distribution was determined using the Student's T-test for independent samples. The statistical significance of the difference was set at $p < 0.05$.

Results and Discussion

In the control **(Table 1, Figures 1a and 1b)**, the cells lay in an even monolayer, tightly adjacent to each other, only single cells were not attached to the substrate. Cells were of prismatic, polygonal, triangular, and rounded shapes. There were single cells of the type of syncytium or symplast with a homogeneous cytoplasm and a shaped nucleus. TCN was 562.66 ± 16.02, while the DCN amount was 8.33 ± 0.44.

Table 1. The effect of selenium nanoparticles on cells in the SPEV-2 culture

Index	Control	Concentration of solution of selenium nanoparticles				
		3.2 μg/mL	32 μg/mL	320 μg/mL	3.2 mg/mL	32 mg/mL

DCN	8.33 ± 0.44	6.16 ± 0.53	9.5 ± 0.75***	17 ± 0.42	300.66 ± 17.34***	316.2 ± 3.58***
TCN	562.66 ± 16.02	446.5 ± 12.32*	392.5 ± 23.16***	427 ± 20.28***	301.33 ± 18.05***	316.2 ± 3.58
DCN/TCN	1.47 ± 0.06	1.39 ± 0.14**	3.13 ± 0.29***	4.03 ± 0.13**	100 ± 0***	100 ± 0***

Note: the differences significantly differ when comparing the values of the experimental and control groups at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***)

At a concentration of a solution of selenium nanoparticles of 3.2 µg/mL, cells were predominantly polygonal, triangular, and rounded in shape, the number of cells not attached to the substrate increased slightly. There were no changes on the part of the cytoplasm and nucleus. TCN was significantly reduced by 20% (446.5 ± 12.32) of the control, which indicated inhibition of cell proliferative activity under the action of selenium nanoparticles [27]. At the same time, there were no significant differences with the control in DCN value **(Table 1, Figures 1c and 1d)**. The luminescence intensity of the nuclei of dead cells was the same as in the control. It should be noted that the change in the color of the medium and the cells themselves occurred due to a solution of selenium nanoparticles.

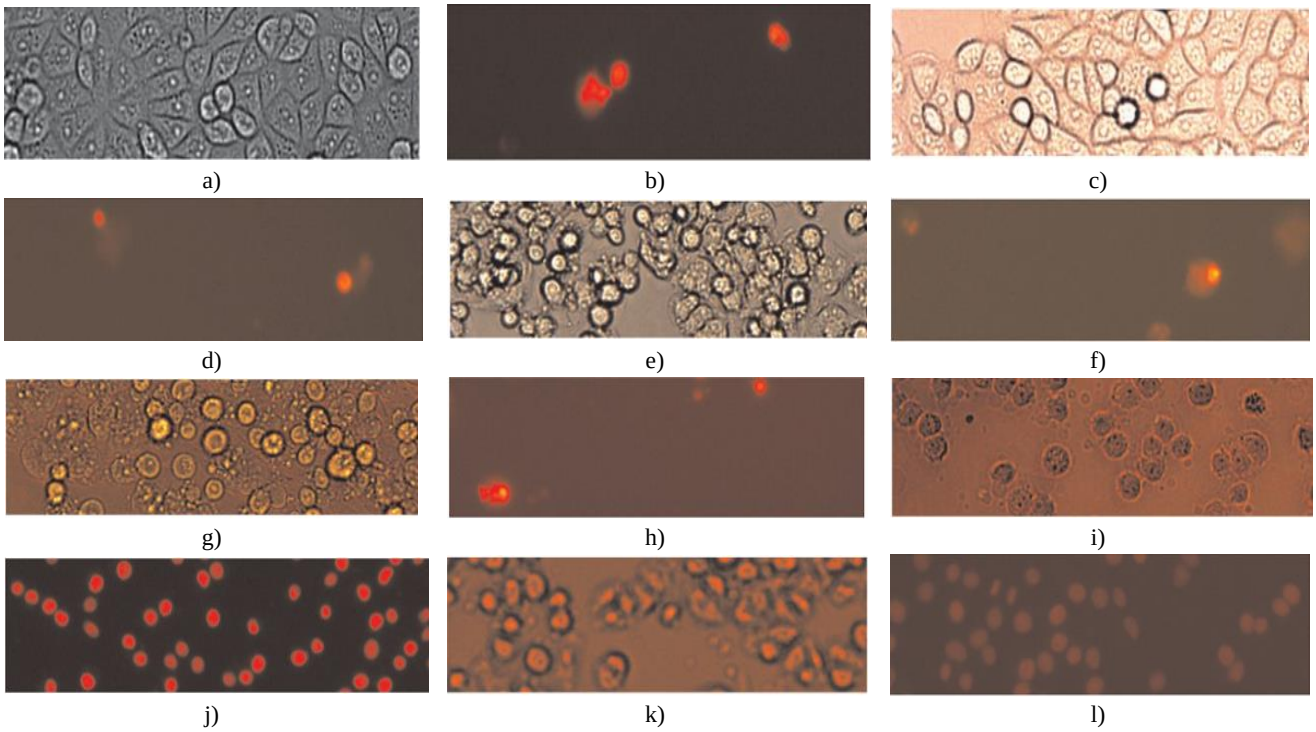


Figure 1. The shape and nature of the arrangement of SPEV-2 culture cells in control (a, b) experimental samples with various concentrations of selenium nanoparticles: 3.2 µg/mL (c, d); 32 µg/mL (e, f); 320 µg/mL (g, h); 3.2 mg/mL (i, j); 32 mg/mL (k, l). Notably, photographs were obtained at light scattering (a, c, e, g, i, k) and fluorescence (b, d, f, h, j, l) modes for each sample.

The superimposition of photographs obtained with different microscopic modes of light scattering and fluorescence registration allowed us to compare the cytomorphological parameters of living and dead cells **(Figures 2 and 3)**. Dead cells with a destroyed membrane, whose DNA was stained with PI, unlike living cells, were detached from the substrate [28, 44]. As can be seen from **Figure 2**, no other cytomorphological differences were found.

At a concentration of 32 µg/mL, there was the appearance of pronounced intracellular granulation, a sharp predominance of rounded cells, mainly with uneven membrane contours. The cells lay less organized and were mostly not attached to the substrate. Single giant cells were rare. TCN was significantly lower than the control by 30% (392.5 ± 23.16), which indicates a significant decrease in their proliferative activity under the action of selenium nanoparticles [20]. DCN was 14% (9.5 ± 0.75) higher than the control **(Table 1)**. The intensity of the glow of the nuclear DNA of the dead cells was the same as at the previous concentration **(Figure 1e)**, which indicates the death of these single cells within a day after the addition of selenium nanoparticles [31]. In addition, single apoptotic corpuscles were found. The intensity of staining of the medium and cells increased with increasing concentration of the solution of selenium nanoparticles **(Figure 1g)**. As a result, at this concentration, a decrease in proliferative activity, and the appearance of cytoplasmic granulation, which is accompanied by cell detachment from the substrate and cell death by apoptosis were observed [45].

At a concentration of 320 µg/mL, the appearance of intracellular granulation, a decrease in cell density and organization, and an increase in their fragmentation were also noted. All the cells were detached from the substrate and had a rounded shape. Single cells had fragmented nuclei, which served as a non-specific sign of the beginning of their death [32]. TCN was 24% (427 ± 20.28) less than the control **(Table 1, Figures 1f and 1h)**, which indicates a decrease in their proliferative activity [43].

DCN, on the contrary, significantly increased by 2 times (17 ± 0.42) **(Table 1)**, which is associated with the cytotoxic effect of selenium nanoparticles [45]. The intensity of the glow of the nuclear DNA of dead cells was pronounced. Apoptotic corpuscles were present. As a result, at this concentration, the previously identified trend persisted: destruction of the membrane of individual detached cells with penetration of the stain into the nucleus and binding to DNA, cell death by apoptosis.

With a 10-fold increase in concentration, with a linear dependence, there should have been a decrease in the number of grown cells several times [46, 47]. Surprisingly, this did not happen in the experiment. On the contrary, at a higher concentration of 320 μg/mL, the number of grown cells was higher than at a concentration of 32 μg/mL **(Table 1)**. Consequently, the degree of proliferation at various concentrations of selenium nanoparticles did not have a linear relationship. Thus, at a concentration of 3.2 μg/mL, pronounced intracellular granulation in the form of dark formations in the cytoplasm was noted. The cells are star-shaped or rounded, but with fragmentation of the membrane and leakage of contents, dead cells formed conglomerates, and cells of the "shadow" type appeared. TCN was 46.4% (301.33 ± 18.05) less than the control **(Table 1, Figures 1j and 1k)**, which indicates a marked decrease in proliferative activity [37]. DCN was 36.5 times higher than the control (300.66 ± 18.05) **(Table 1)** and significantly differed in comparison with the previous concentration of the solution of selenium nanoparticles. In addition, the DCN is almost identical to the TCN. The intensity of the glow of the dead cells was more pronounced than in the control, which indicates their loss directly during the experiment during the day [29]. Interestingly, no apoptotic bodies were detected. Apparently, during the first day, selenium nanoparticles in high concentration do not contribute to the activation of apoptosis mechanisms in the cell but lead to their mass death due to direct cytotoxic action [30, 48].

At a concentration of 32 mg/mL, a change like intracellular granulations was noted: the number of granules in the cytoplasm and their sizes increased. The cell nuclei did not differentiate. The cells acquired a stellate shape and less often retained a rounded shape **(Figure 2)**.

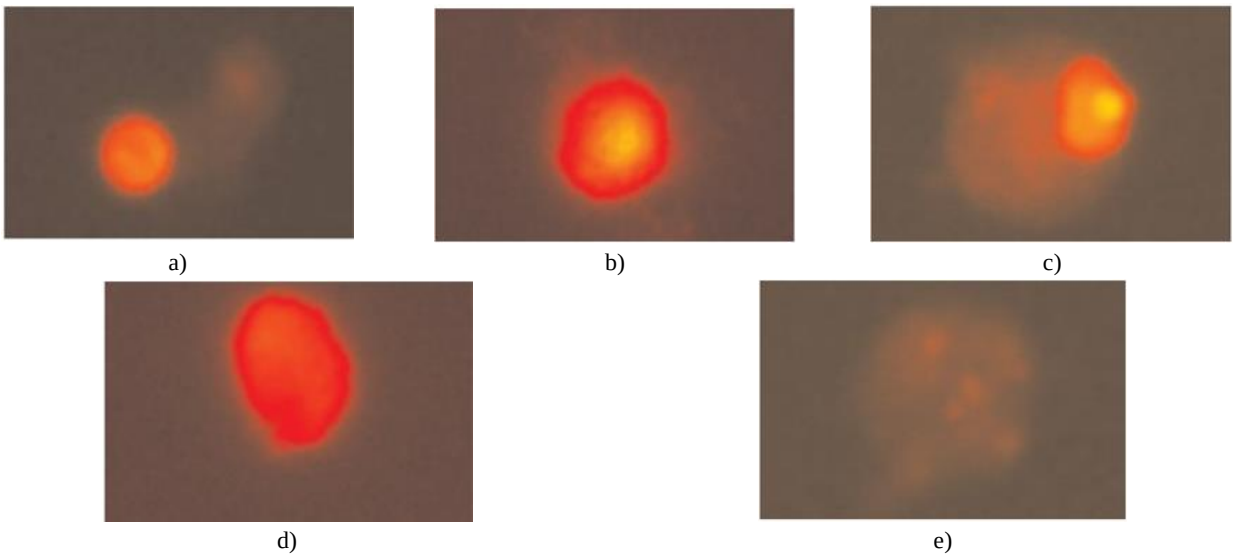
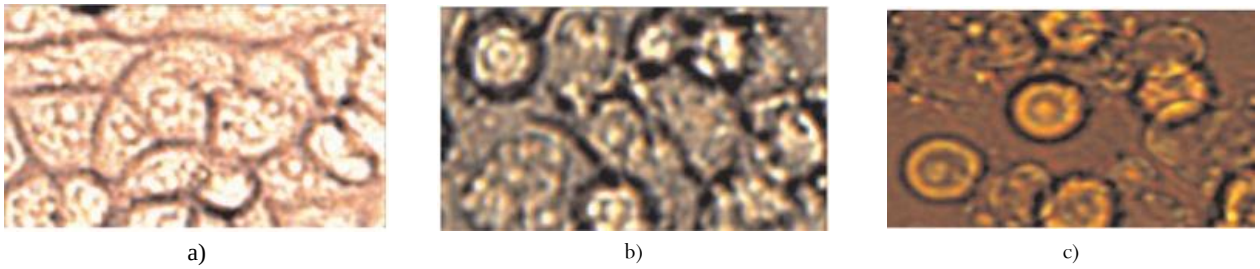
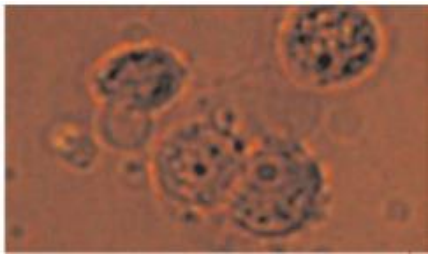


Figure 2. The stages of damage to the DNA of the nucleus, depending on the degree of damage to the cell, the binding of the stain to the DNA of the nucleus, and the time from the onset of death of the propidium-positive cell obtained by fluorescence microscopy: a) 3.2 μg/mL; b) 32 μg/mL; c) 320 μg/mL; d) 3.2 μg/mL; e) 32 μg/mL

Notably, DCN (316.2 ± 3.58) coincided with TCN, which indicates 100% of their death. Compared with the control, the DCN significantly increased by 38 times. Some of the cells were in a state of swelling, apparently due to the influx of a solution of selenium nanoparticles through a destroyed membrane, while others showed fragmentation of the cell membrane with leakage of contents. The conglomeration of dead cells was more pronounced, due to an increase in their number in conglomerates compared to the previous concentration **(Figure 3)**.





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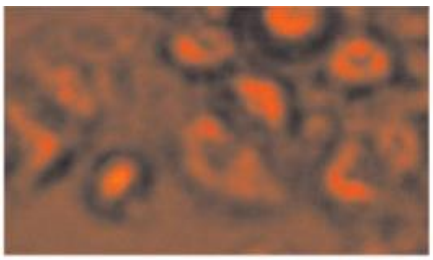

e)

Figure 3. The stages of damage to the DNA of the nucleus, depending on the degree of damage to the cell, the binding of the stain to the DNA of the nucleus, and the time from the onset of death of the propidium-positive cell obtained by light microscopy: a) 3.2 μg/mL; b) 32 μg/mL; c) 320 μg/mL; d) 3.2 μg/mL; e) 32 μg/mL

TCN was 43% less than the control (316.2 ± 3.58) **(Table 1, Figures 1k and 1l)**. The luminescence intensity of the dead cells was much lower than at the previous concentration. This can be explained by a denser medium with a high concentration of selenium nanoparticles [49, 50]. In general, at a concentration of 3.2 mg/ml, we revealed the same trend: mass cell death by necrosis, without signs of apoptosis.

Conclusion

The analysis of cytomorphological changes in the SPEV-2 culture was performed in parallel with the determination of the dead cells number, which allowed us to identify the peculiarities of the effect of selenium nanoparticles at different concentrations and to determine the effect of these concentrations on the morphophysiological parameters of cells. It is worth noting that all concentrations of selenium nanoparticles had a cytostatic effect, suppressing the proliferative activity of cells. At the same time, the linear dependence of the decrease in total cell number on the increase in concentration has not been established. The minimum concentration of a solution of selenium nanoparticles, which reliably has a cytotoxic effect on SPEV-2 cells in culture is 32 μg/mL. The maximum cytotoxicity had a solution of selenium nanoparticles at a concentration of 3.2 mg/mL.

It was found that concentrations such as 32 and 320 μg/mL had apoptotic activity. While high concentrations (3.2 and 32 mg/ml) caused cell necrosis. At relatively low concentrations (32 and 320 μg/mL) cells were detached from the substrate and their contents were granulating, which indicates a deterioration in their condition as a whole. After that, the mechanisms of apoptosis are activated in individual cells.

A feature of cells that died as a result of necrosis during microscopy in phase contrast is the appearance of dark formations in the cytoplasm, the fuzzy contours of the cells themselves and their acquisition of a stellate shape or preservation of a rounded one, but with fragmentation of the cell membrane and leakage of contents. At high concentrations of selenium nanoparticles, dead cells form conglomerates and cell swelling occurs. A decrease in the luminescence intensity of propidium iodide indicates the decay of the DNA of the nucleus, which is shown in **Figures 2 and 3**. The DCN index can be considered as an indicator of cytotoxicity.

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Conflict of interest: None

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Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

References

1. Liang J, He Y, Huang C, Ji F, Zhou X, Yin Y. The regulation of selenoproteins in diabetes: A new way to treat diabetes. Curr Pharm Des. 2024;30(20):1541-7. doi:10.2174/0113816128302667240422110226
2. Yuan S, Zhang Y, Dong PY, Chen Yan YM, Liu J, Zhang BQ, et al. A comprehensive review on the potential role of selenium, selenoproteins and selenium nanoparticles in male fertility. Heliyon. 2024;10(15):e34975. doi:10.1016/j.heliyon.2024.e34975
3. Nisa S, Fatima M, Shah SZH, Khan N, Aftab B, Ali W, et al. Evaluation of growth, antioxidant status, hepatic enzymes and immunity of Nanoselenium-Fed Cirrhinus mrigala. PLoS One. 2024;19(8):e0308761. doi:10.1371/journal.pone.0308761
4. Ding H, Lu X, Ji X, Wang S, Jin J, Zhao M, et al. Synthesis of glucosamine-selenium compound and evaluation of its oral toxicity and in vitro anti-hepatitis B virus activity. Chem Biol Interact. 2024;402:111184. doi:10.1016/j.cbi.2024.111184
5. Sun X, Zhang W, Shi X, Wang Y, Zhang Y, Liu X, et al. Selenium deficiency caused hepatitis in chickens via the miR-138-5p/SelM/ROS/Ca2+ overload pathway induced by hepatocyte necroptosis. Food Funct. 2023;14(20):9226-42. doi:10.1039/d3fo00683b
6. Jia MJ, Chen L. Effect of trace elements and nutrients on diabetes and its complications: A mendelian randomization study. Front Nutr. 2024;11:1439217. doi:10.3389/fnut.2024.1439217

7. Liang Q, Huang R, Peng Z, Zou M. Impact of dietary selenium and blood concentration on liver function: A population-based study. Front Nutr. 2024;11:1415288. doi:10.3389/fnut.2024.1415288
8. Gallo-Rodriguez C, Rodriguez JB. Organoselenium compounds in medicinal chemistry. ChemMedChem. 2024:e202400063. doi:10.1002/cmdc.202400063
9. Leszto K, Biskup L, Korona K, Marcinkowska W, Możdżan M, Węgiel A, et al. Selenium as a modulator of redox reactions in the prevention and treatment of cardiovascular diseases. Antioxidants (Basel). 2024;13(6):688. doi:10.3390/antiox13060688
10. Pyka P, Garbo S, Fioravanti R, Jacob C, Hittinger M, Handzlik J, et al. Selenium-containing compounds: A new hope for innovative treatments in Alzheimer's disease and Parkinson's disease. Drug Discov Today. 2024;29(8):104062. doi:10.1016/j.drudis.2024.104062
11. Shi H, Wang B, Ma H, Li Y, Du J, Zhang B, et al. Preparation of biomimetic selenium-baicalein nanoparticles and their targeted therapeutic application in nonsmall cell lung cancer. Mol Pharm. 2024;21(9):4476-89. doi:10.1021/acs.molpharmaceut.4c00390
12. Jiang H, Yang G, Chen J, Yuan S, Wu J, Zhang J, et al. The correlation between selenium intake and lung function in asthmatic people: A cross-sectional study. Front Nutr. 2024;11:1362119. doi:10.3389/fnut.2024.1362119
13. Zhuang Y, Wang Y, Sun P, Ke J, Chen F. Association between blood lead, cadmium, selenium levels and hyperlipidemia: A population-based study. PLoS One. 2024;19(8):e0306573. doi:10.1371/journal.pone.0306573
14. Ikeda A, Marsela M, Miyashita C, Yamaguchi T, Saijo Y, Ito Y, et al. Heavy metals and trace elements in maternal blood and prevalence of congenital limb abnormalities among newborns: The Japan environment and children's study. Environ Health Prev Med. 2024;29:36. doi:10.1265/ehpm.23-00366
15. Chung CW, Kim K, Park SK, Ju DL, Park YJ, Shin CH, et al. Selenium levels and their association with thyroid autoimmunity and severe preeclampsia in pregnancy: Insights from a prospective ideal breast milk cohort study. Eur Thyroid J. 2024;13(4):e240007. doi:10.1530/ETJ-24-0007
16. Yavari M, Akbari M, Ramezani Ahmadi A, Siavash Dastjerdi M, Hashemi MS. Investigating the effect of combined use of selenium and Myo-inositol supplements on thyroid function and autoimmune characteristics in thyroid disorders: A systematic review and meta-analysis. Expert Rev Endocrinol Metab. 2024;19(3):269-77. doi:10.1080/17446651.2023.2295487
17. Altmayer LA, Lang M, Schleicher JT, Stuhlert C, Wörmann C, Scherer LS, et al. A Plea for monitoring serum selenium levels in breast cancer patients: Selenium deficiency is rare during the first year of therapy, and selenium supplementation is associated with elevated risk of overdosing. Nutrients. 2024;16(13):2134. doi:10.3390/nu16132134
18. Wang K, Shi X, Lin H, Xu T, Xu S. Selenium deficiency exacerbates ROS/ER stress mediated pyroptosis and ferroptosis induced by bisphenol A in chickens thymus. J Environ Sci (China). 2025;148:13-26. doi:10.1016/j.jes.2024.01.002
19. Darbani Torshizi M, Younesian O, Aboomardani M, Roshandel G, Hosseinzadeh S, Hosseini Alarzi SS, et al. Serum Selenium, Vitamin A, and Vitamin E levels of healthy individuals in high- and low-risk areas of esophageal cancer. Middle East J Dig Dis. 2022;14(4):396-403. doi:10.34172/mejdd.2022.300
20. Deshmukh R, Singh R, Sharma S, Mishra AK, Harwansh RK. A Snapshot of selenium-enclosed nanoparticles for the management of cancer. Curr Pharm Des. 2024;30(11):841-58. doi:10.2174/0113816128297329240305071103
21. Chalcarz M, Grabarek BO, de Mezer M, Krokowicz P, Patera J, Czupryna K, et al. Serum selenium concentration as a potential diagnostic marker for early-stage colorectal cancer: A comparative study. Med Sci Monit. 2024;30:e942882. doi:10.12659/MSM.942882
22. Shao C, Luo T, Wang S, Li Z, Yu X, Wu Y, et al. Selenium nanoparticles alleviates cadmium induced hepatotoxicity by inhibiting ferroptosis and oxidative stress in vivo and in vitro. Chemosphere. 2024;364:143004. doi:10.1016/j.chemosphere.2024.143004
23. Nagdalian AA, Blinov AV, Siddiqui SA, Gvozdenko AA, Golik AB, Maglakelidze DG, et al. Effect of selenium nanoparticles on biological and morphofunctional parameters of barley seeds (Hordéum vulgáre L.). Sci Rep. 2023;13(1):6453. doi:10.1038/s41598-023-33581-6
24. Nag S, Kar S, Mishra S, Stany B, Seelan A, Mohanto S, et al. Unveiling green synthesis and biomedical theranostic paradigms of selenium nanoparticles (SeNPs) - A state-of-the-art comprehensive update. Int J Pharm. 2024;662:124535. doi:10.1016/j.ijpharm.2024.124535
25. Johnson J, Shanmugam R, Manigandan P. Characterization and biomedical applications of green-synthesized selenium nanoparticles using tridax procumbens stem extract. Cureus. 2024;16(6):e63535. doi:10.7759/cureus.63535
26. Mendes C, Lemos I, Hipólito A, Abreu B, Freitas-Dias C, Martins F, et al. Metabolic profiling and combined therapeutic strategies unveil the cytotoxic potential of selenium-chrysin (SeChry) in NSCLC cells. Biosci Rep. 2024;44(7):BSR20240752. doi:10.1042/BSR20240752
27. Blinov AV, Maglakelidze DG, Rekhman ZA, Yasnaya MA, Gvozdenko AA, Golik AB, et al. Investigation of the effect of dispersion medium parameters on the aggregative stability of selenium nanoparticles stabilized with catamine AB. Micromachines (Basel). 2023;14(2):433. doi:10.3390/mi14020433
28. Zhang L, Liu H, Shen J, Liu W, Liu D, Cheng L, et al. Selenium nanoparticles enhance the anti-tumor immune responses of anti-4-1BB antibody and alleviate the adverse

effects on mice. Immunobiology. 2024;229(5):152839. doi:10.1016/j.imbio.2024.152839

29. Derakhshan-Sefidi M, Bakhshi B, Rasekhi A. Thiolated chitosan nanoparticles encapsulated nisin and selenium: Antimicrobial/antibiofilm/anti-attachment/immunomodulatory multi-functional agent. BMC Microbiol. 2024;24(1):257. doi:10.1186/s12866-024-03400-7
30. Blinova A, Blinov A, Kravtsov A, Nagdalian A, Rekhman Z, Gvozdenko A, et al. Synthesis, characterization and potential antimicrobial activity of selenium nanoparticles stabilized with cetyltrimethylammonium chloride. Nanomaterials (Basel). 2023;13(24):3128. doi:10.3390/nano13243128
31. Turabov ME, Kolesnikova VV, Bazaeva AR, Dzukheraeva DB, Ternovoy VV, Kompaneec AD, et al. Evaluation of cytotoxic and cytostatic activity of chlorophytum comosum methanol extract. Pharmacophore. 2024;15(4):33-8. doi:10.51847/D5CauwttEJ
32. Dengler WA, Schulte J, Berger DP, Mertelsmann R, Fiebig HH. Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assays. Anticancer Drugs. 1995;6(4):522-32. doi:10.1097/00001813-199508000-00005
33. Shekatkar M, Kheur S, Deshpande S, Sakhare S, Kumbhar G, Kheur M, et al. Estimation of salivary magnesium levels in patients with oral squamous cell carcinoma. Clin Cancer Investig J. 2022;11(3):30-4.
34. Babalghith AO. Coenzyme Q10 regulates gene expression of myocardial infarction in isoproterenol model. Arch Pharm Pract. 2022;13(2-2022):1-6.
35. Piccinini F, Tesei A, Arienti C, Bevilacqua A. Cell counting and viability assessment of 2D and 3D cell cultures: Expected reliability of the trypan blue assay. Biol Proced Online. 2017;19:8. doi:10.1186/s12575-017-0056-3
36. Krishnamurthy S, Ban K. Feasibility of using digital confocal microscopy for cytopathological examination in clinical practice. Mod Pathol. 2022;35(3):319-25. doi:10.1038/s41379-021-00925-4
37. Zhu D, Wang H, Wu W, Geng S, Zhong G, Li Y, et al. Circulating cell-free DNA fragmentation is a stepwise and conserved process linked to apoptosis. BMC Biol. 2023;21(1):253. doi:10.1186/s12915-023-01752-6
38. Wu Q, Wang X, Hao S, Wu Y, Zhang W, Chen L, et al. Synergetic effects and inhibition mechanisms of the polysaccharide-selenium nanoparticle complex in human hepatocarcinoma cell proliferation. J Sci Food Agric. 2024;104(9):5124-38. doi:10.1002/jsfa.13335
39. Jiwangga D, Mastutik G, Mahyudin F, Meitavany EN, Juliana, Dwipayana MA, et al. In vitro enhancement of chondrogenic and epithelial genes differentiation of human adipose mesenchymal stem cells in decellularized xenograft tracheal scaffold: Implications for tracheal disease management. J Med Pharm Chemi Res. 2024;7:393-409. doi:10.48309/jmpcr.2025.464103.1301
40. Sadulaev R, Magomedov T, Khurtueva A, Geteriev A, Turabov N, Koba A, et al. Toxicological assessment of the effect of cadmium chloride on quantitative and qualitative parameters of spermatogenesis in vivo. J Med Pharm Chem Res. 2025;7(2):321-32. doi:10.48309/jmpcr.2025.458716.1257
41. Yan YQ, Liu M, Xu ZJ, Xu ZJ, Huang YX, Li XM, et al. Optimum doses and forms of selenium maintaining reproductive health via regulating homeostasis of gut microbiota and testicular redox, inflammation, cell proliferation, and apoptosis in roosters. J Nutr. 2024;154(2):369-80. doi:10.1016/j.tjnut.2023.12.021
42. Yu H, He X, Gu X, Hou Y, Zhao H, Gao L, et al. Carbon-coated selenium nanoparticles for photothermal therapy in choriocarcinoma cells. RSC Adv. 2024;14(1):640-9. doi:10.1039/d3ra07085a
43. He Z, Song H, Cheng J, Qiu W, Hu J, Qiu Z, et al. Selenium nanoparticles stabilized by β-glucan nanotubes from black fungus and their effects on the proliferation, apoptosis, and cell cycle of HepG2 cells. ACS Omega. 2023;8(48):45358-68. doi:10.1021/acsomega.3c04244
44. Almalki GH, Rabah S, Arafa NM, Bahshwan SM. Immunohistochemical evaluation of the euphorbia inarticulata extract on liver and kidney tissues in hepatocellular carcinoma rats. Pharmacophore. 2022;13(2-2022):33-40.
45. Miere FG, Ganea M, Teodorescu AG, Fritea L, Lestyan M, Horvath T, et al. The green synthesis of silver and selenium nanoparticles using the plant stellaria media (L.) vill. Pharmacophore. 2022;13(2-2022):88-95.
46. Sohal KS, Owibingire SS, Moshy JR, Deoglas DK, Laizer PJ, Kalyanyama BM, et al. Orofacial squamous cell carcinoma: Analysis of histopathological reports of 465 patients in Tanzania. Clin Cancer Investig J. 2022;11(3):9-14.
47. Dal MB. Surgical treatment results in obstetric and iatrogenic anal sphincter injuries. Arch Pharm Pract. 2022;13(1-2022):58-60.
48. Li Y, Liu M, Kong B, Zhang G, Zhang Q. The role of selenium intervention in gut microbiota homeostasis and gene function in mice with breast cancer on a high-fat diet. Front Microbiol. 2024;15:1439652. doi:10.3389/fmicb.2024.1439652
49. Wang Z, Ma H, Song Y, Lin T, Liu L, Zhou Z, et al. Plasma selenium and the risk of first stroke in adults with hypertension: A secondary analysis of the China stroke primary prevention trial. Am J Clin Nutr. 2022;115(1):222-31. doi:10.1093/ajcn/nqab320
50. Peng D, Zhang J, Liu Q, Taylor EW. Size effect of elemental selenium nanoparticles (Nano-Se) at supranutritional levels on selenium accumulation and glutathione S-transferase activity. J Inorg Biochem. 2007;101(10):1457-63. doi:10.1016/j.jinorgbio.2007.06.021