Effect of Selenium Nitrite Feeding for 28 Days on Food Intake and Growth of Rats

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Abstract: This experiment was aimed at verifying the 28 day oral toxicity test (GB 15193.22-2014) in accordance with the national food safety standard [11]. Rats were fed with a high amount in selenium nitrite for 28 consecutive days to observe its effect on food intake and growth of rats, and at the same time provide a basis for predicting the safety of selenium preparations consumed by the population and long-term consumption. The detection indicators included general condition, body weight, food intake, food utilization rate, and pathological examination. During the entire observation period, the animals in each dose group were generally in good condition, and no clinical symptoms related to the test substance were seen. , showed a dose-dependent inhibition on the growth of body weight and food intake of male animals, especially the growth of animals in the high-dose group was significantly inhibited, and its body weight was significantly lower than that of the control group ($P<0.01$ or $P<0.05$), and had no significant effect on food utilization. The effect on the body weight of female animals is bidirectional. The body weight of the animals in the middle-dose group increased significantly, while that in the high-dose group decreased significantly, and there was no significant change in the low-dose group. Finally, it was shown that the intervention of Selenium nitrite in the high-dose group would affect the body weight change of SPF Sprague-Dawley rats.

1. Introduction

As a naturally occurring metalloid element, selenium (Se) is vital for human and animal health. Se is the raw material for the synthesis of 25 kinds of selenoproteins containing selenocysteine (Sec) active groups in the body, it supports the antioxidant defense systems by being integrated into selenoproteins, which have neuroprotective effects, regulate reproductive processes and assists in the metabolism of thyroid hormones[1,2]. Among all the essential nutrients to the human body, the gap between dietary shortage and hazardous levels for Se is relatively shorter, given that inorganic and organic species have different biological characteristics, and their level of toxicity could vary depending on their chemical form.

Optimization of population Se intake has been a critical topic in contemporary healthcare globally in the last few decades. Studies have shown that selenium presents a U-shaped toxic effect, that is, too low or too high selenium intake will cause harm to the body. Low serum selenium concentrations are associated with impaired immune function, cognitive decline, and increased mortality[1][3], typical deficiencies are Keshan and Kashin-Beck diseases[1], manifested by muscle pain, cardiovascular problems, decreased immune system function and thyroid problems, usually in areas with selenium-deficient soils. Excessive selenium intake can lead to damage to the heart, liver, kidney and other organs as well as metabolic disorders, including relatively mild symptoms including brittle nails, discolored teeth, skin lesions, and hair loss. In severe cases, poisoning symptoms include diarrhoea, abdominal pain, nausea, vomiting, liver and kidney damage, etc[1][3]. This suggests that in the process of supplementing selenium preparations, it is very important to choose the appropriate dose of selenium.

The recommended nutrient intake (RNI) and upper intake (UL) of selenium are critical in selenium supplementation. According to the study of the total intake of multiple nutrients in the Chinese general population, the selenium intake standard for adults (over 18 years old) is 60ug/day for RNI and 400ug/day for UL[4,5]. In 2000, the Scientific Committee on Food (SCF) determined the UL for adults, including pregnant and lactating women, to be 300 μg/day based on evidence from observational studies of long-term exposure to selenium, followed by some authoritative organizations such as the National Health and Medical Research Council (NHMRC) and World Health Organization (WHO) raised the UL data to 400u g/day[6].

Therefore, healthy selenium supplementation is a low-to-medium dose, and the selenium element only needs to be supplemented through drugs and food[3]. Food is the main source of selenium. Some selenium-rich foods include nuts, selenium yeast, seafood (such as shellfish, crab, and fish), meat (such as chicken, beef, and pork), and some grains (such as oats, wheat, and barley). Intake of

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these selenium-rich foods through a balanced diet can meet the selenium needs of normal people. Food supplementation is the most common, natural and safe way to ingest selenium. Pharmaceutical supplements such as selenium supplements are a form that provides high concentrations of selenium either by oral or parenteral routes[3]. These supplements are often provided in the form of selenium compounds such as selenium yeast, selenate, or selenomethionine. However, selenium supplements are usually used to treat selenium deficiency or adjuvant treatment of certain diseases[3], so food selenium supplementation should be the first choice.

2. Materials and methods

2.1. Grouping of experimental animals

The rats used in this experiment were Sprague-Dawley rats that purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. 200 rats were randomly grouped according to body weight, as shown in Table 1. The animals in the main experimental group were divided into a negative control group, low-dose group, medium-dose group and high-dose group, 44 animals were in each group (22 male and 22 male), and satellite groups were set up in the negative control group and high dose group, 12 rats in each group (6 male and 6 females respectively). This experiment complied with the experimental protocol, the GLP management requirements and related standard operating procedures (SOPs), and the experiment was conducted by OECD guideline 407[7] (Repeated dose 28-day oral toxicity study in rodents).

Table 1. Grouping of experimental animals

Number	Group Classification	Group Type	Selenium nitrite Content in Feed	Selenium content in the feed	Theoretical dose (based on selenium content) (Mg Se/kg)	*Actual dosage for male Rats (Mg Se/kg)	*Actual dosage for male Rats (Mg Se/kg)	Number of animals
1	Se- N1	Normal feed group - common	——	0.2 ppm	0.010	0.007	0.013	28 F/28 M
2	Na_2SeO_3-L	Selenium nitrite low-dose group	1.533 ppm	0.7 ppm	0.035	0.051	0.060	22 F/22 M
3	Na_2SeO_3-M	Selenium nitrite middle dose group	4.599 ppm	2.1 ppm	0.105	0.103	0.115	22 F/22 M
4	Na_2SeO_3-H	Selenium nitrite high-dose group	13.797 ppm	6.3 ppm	0.315	0.294	0.356	28 F/28 M

*The actual selenium intake of the animal is calculated based on the actual selenium content of the feed, animal food intake and animal weight. The molecular weight of Na2SeO3 is 172.94, and Se accounts for 78.96/172.94=45.66%. 1ppm means 1mg of the target substance in 1kg of feed

2.2. Feed Preparation and selenium intervention

Carry out the preparation of high, middle and low dose group feeds according to the table 2. Animals in corresponding groups were fed continuously with the prepared feed for 4 weeks. The sodium selenite used for preparaing selenium nitrite was purchased from Sigma Aldrich (China) Company.

Table 2. Feed Preparation and selenium intervention

Feed number	Group Classification	Groups	Selenium nitrite Content in Feed	Selenium content in the feed	Feed Formula	
					Amount of Selenium nitrite added (corrected after purity 98%) mg	Maintenance feed
1	Control	Normal feed group - common	——	0.2 ppm	0	60kg
2	Na_2SeO_3-L	Selenium nitrite low-dose group	1.533ppm	0.7 ppm+0.2 ppm	93.9mg	60kg
3	Na_2SeO_3-M	Selenium nitrite middle dose group	4.599ppm	2.1 ppm+0.2 ppm	281.6mg	60kg
4	Na_2SeO_3-H	Selenium nitrite high-dose group	13.797ppm	6.3 ppm+0.2 ppm	844.7mg	60kg

2.3. Sample Content Confirmation and Uniformity

From the prepared feed packaging bags of each dosage group, the total selenium content was detected after sampling at three points (upper, middle and lower). The contents of all samples collected at 3 points were between (85%) and (115%) of the theoretical value, and the relative standard deviation (RSD) was less than 10%.

2.4. Observation Indicators and Methods

2.4.1 Viability and clinical observation

From the arrival of the animals until the day of animal dissection, the survival (dying and death) and symptom observations were carried out once a day. Detailed records of animal clinical manifestations, signs, degree and duration of intoxication, observation of coat, skin, mucous membranes, secretions, excretions, respiratory system, nervous system, autonomic activities (such as tearing, piloerection, pupil size, abnormal breathing), as well as the time, size, and location of the tumor.

2.4.2 Eye examination

Eye examinations (cornea, lens, bulbar conjunctiva, iris) were performed before and at the end of the experiment.

2.4.3 Body weight, food intake and food utilization

Body weight was measured once a week. The measurement method is to weigh all the animals using an electronic balance and calculate the weight gain of the animals at the same time. Weigh the weekly food intake once a week (weigh the added amount on the 1st day and weigh the remaining amount on the 8th day), record the added amount and the remaining amount, and calculate the daily food intake of each group. Finally, calculate weekly food utilization and total food utilization.

2.4.4 Gross autopsy histopathological examination

Necropsy was performed on animals found dead or euthanized before administration, and tissue preservation was determined by the subject leader. The unplanned and planned animals were first anaesthetized by intramuscular injection of Mianling II injection, then bled (collecting blood samples) for euthanasia, and then necropsied as soon as possible.

2.5. Statistical analysis

The following does not apply to unplanned experimental data, which will be reported separately. Unless otherwise stated, all numerical data such as body weight, food intake, organ weights, clinical parameters and other quantitative data collected according to the experimental protocol were calculated as group means and standard deviations.

The mean values were compared with the control group using the following statistical methods. Statistical descriptions are made by analysis variables and categorical variables (such as gender, measurement time, and other variables that can be subdivided into statistical descriptions) and added to the corresponding data tables. Data sets with less than three non-missing values will not be subjected to the following statistical analysis. Statistical analysis was performed on females and males using SPSS software. When there were more than two groups, the Levene test for homogeneity of variance was performed. If the homogeneity of variance analysis was not significant, that is, $P>0.05$, a one-way analysis of variance (ANOVA) was used. When the analysis of variance is significant, that is, $P\leq0.05$, the Dunnett-t test is used to compare the treatment groups of each test product with the control group. If the Levene test $P\leq0.05$, the Dunnett-T3 test was used for comparison between groups.

The hypothesis test adopts a two-sided test, and the test level is 0.05. Significance was identified as $P\leq0.05$ or $P\leq0.01$, where P is the obtained probability value.

2.6. Animal Welfare

The institution fully complies with the relevant national regulations on the welfare of experimental animals, and the use of experimental animals has been approved by the "Animal Use and Management Committee" of the institution. (Approval number: Welfare number: Safety Evaluation Center Action (Fu) No. 202022005).

3. Results

3.1. Selenium content and homogeneity analysis

The relative standard deviations of the selenium content measured at 3-4 points of the feed in the three dosage groups were all less than 10%, which met the requirements. The selenium content of each batch of feed was different from the theoretical value, see table 3 for details. The actual dose of this experiment will be determined according to the actual exposure calculated from the average value of the detected selenium content and the amount of food consumed.

Table 3. Selenium content test results

Feed batch	Selenium content (ppm)										
	Control	sampling location	Low dose	Average	Standard deviation	Middle dose	Average	Standard deviation	High dose	Average	standard deviation
200012049	0.4	1	0.84	0.81	0.05	1.67	1.63	0.16	4.33	4.21	0.35
		2	0.84			1.45			3.82		
		3	0.76			1.76			4.49		
		4	0.94			1.84			8.16		

3.2. Clinical observation

During the entire observation period, the animals grew generally well, and no death or dying was found, and no other abnormalities, such as hair loss, emaciation, and lumps, were found.

3.3. Eye examination

No obvious abnormalities were found in the ophthalmic examinations (cornea, lens, bulbar conjunctiva, iris) of all rats before the experiment; no obvious abnormalities were found in the ophthalmic examinations (cornea, lens, bulbar conjunctiva, iris) of the negative control group and high-dose rats at the end of the experiment.

3.4. Effects of food intake, food Utilization and body Weight

3.41 Effect on food intake

See figure 1 and 2 for details. Compared with the negative control group in the same period, the food intake of female and male high-dose groups decreased significantly from the first week of feeding selenium-containing feed ($P<0.05$ or $P<0.01$). However, the food intake of the medium-dose group did not change in line with the increase in body weight. Compared with the negative control group, the food intake of the medium-dose group did not increase significantly.

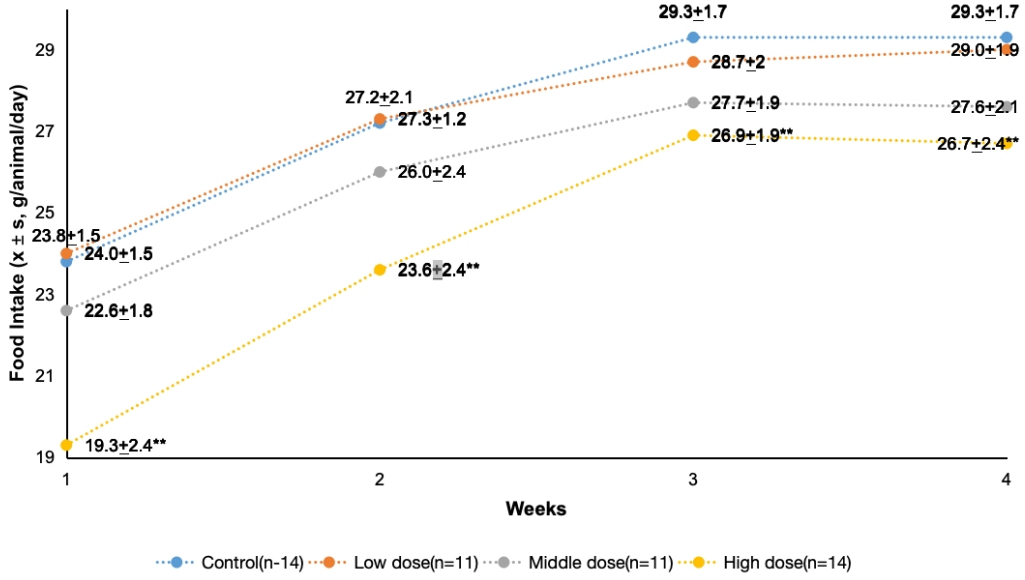

Figure 1. Changes in food intake of rats intervened with different doses of selenium (female) (x ± s, g/animal/day)

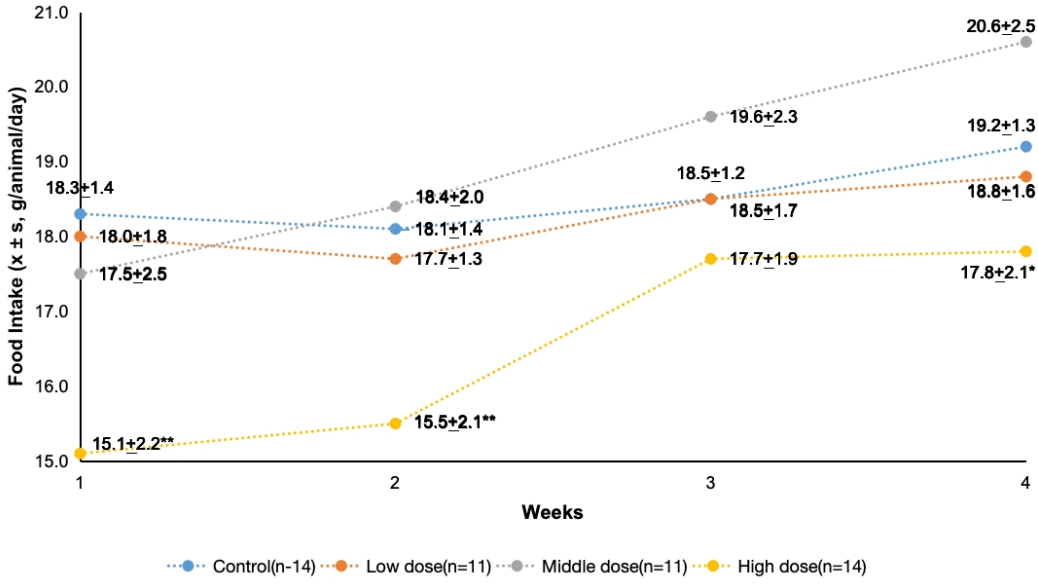

Figure 2. Changes in food intake of rats intervened with different doses of selenium (male) (x ± s, g/animal/day)

3.4.2 Impact on food utilization

See figure 3 and figure 4 for details. Compared with the negative control group, the food utilization rate of male rats did not change significantly, and the food utilization rate of female rats in the high-dose group decreased, especially in the 2nd, 3rd, and 4th weeks, but only the 2nd week The difference was significant ($P<0.05$ or $P<0.01$).

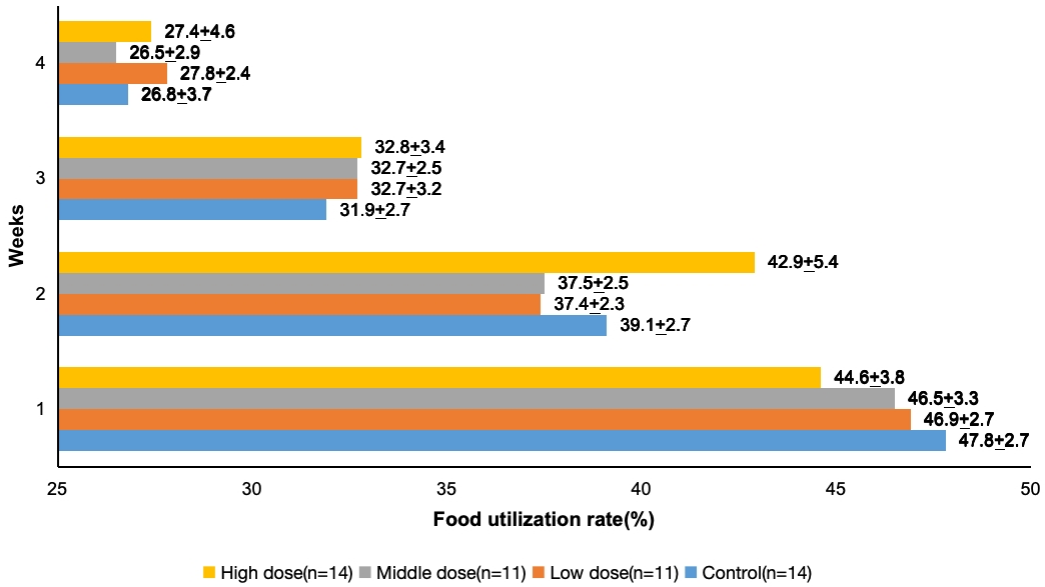

Figure 3. Food utilization rate of rats intervened with different doses of selenium (male)

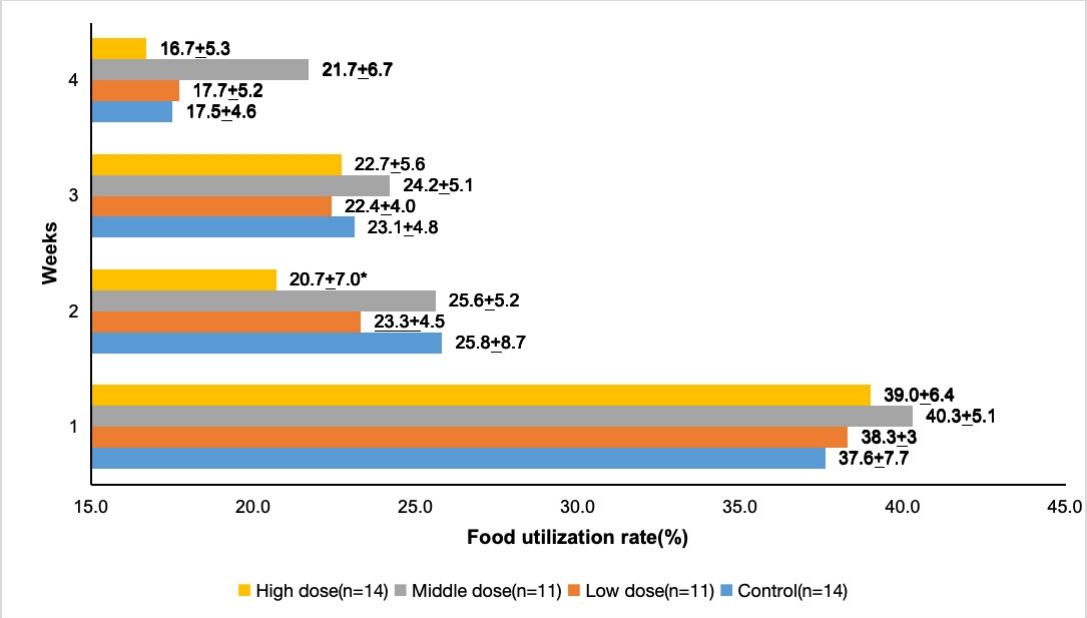

Figure 4. Food utilization rate of rats intervened with different doses of selenium (female)

3.4.3 Effect on body weight

Referring to attached table 4 and table 5. The test samples inhibited the growth of male animal body weight in a dose-dependent manner. Compared with the control group, the body weight of the animals in the high-dose group (since the first week of feeding selenium-containing feed) and the total weight gain of the animals in the high-dose group were significantly reduced (P<0.05 or P<0.01). The body weight and total weight gain of the middle dose group were significantly higher than those of the control group in the fourth week (P<0.05 or P<0.01), while the body weight and total weight gain of the high dose group were significantly higher during the same period. decreased (P<0.05 or P<0.01).

Table 4. Changes in body weight of rats intervened with different doses of selenium (male, x ± s, g)

Group/Weels	Control (n=28)	Low dose (n=22)	Middle dose (n=22)	High dose (n=28)
0	121.6±6.2	121.9±6.9	121.9±6.8	121.7±6.3
1	212.8±13.4	212.0±10.7	205.9±12.8	190.7±13.9**
2	287.6±21.4	283.5±14.7	274.2±18.6**	261.0±18.1**
3	352.9±26.5	349.4±21.0	337.5±24.2** 88	322.7±20.7**
4	408.2±35.4	406.1±27	388.8±29.4**	374.4±27.4**

Table 5. Changes in body weight of rats intervened with different doses of selenium (female, x ± s, g)

Group/Weels	Control (n=28)	Low dose (n=22)	Middle dose (n=22)	High dose (n=28)
0	113.0±5.9	113.2±6.6	113.1±6.5	113.1±5.9
1	168.5±13.7	168.4±13.1	169.5±13.0	159.9±10.3*
2	201.2±13.1	197.3±16.2	202.4±14.7	183.1±15.3**
3	231.3±15	226.7±20.3	236.0±21.7	211.4±19.6**
4	254.9±17	250.1±21.9	267.8±29.3*	232.6±23.8**

4. Discussion

According to the results, it can be seen that no abnormalities were found in the selenium content test results and homogeneity analysis, clinical observation and eye examination, indicating that the feed with higher selenium content has no significant impact on these indicators. However, anomalies emerged in three indicators of food intake, food utilization, and body weight, which need to be further discussed.

In the results of the effect of food intake (figure 1&2), the food intake of the medium and low dose groups did not change with the increase in body weight. Compared with the negative control group in the same period, the food intake of the female and male high-dose groups fed with selenium-containing feed decreased significantly in the first week. This is mainly due to the toxic effects of ingesting high doses of selenium[1], as mentioned in the introduction section gastrointestinal toxicity]8] and hepatotoxicity[8] of excess selenium, causing toxic symptoms such as abdominal pain, nausea, vomiting, etc., which may suppress the appetite of Rats. Food intake is the main cause affecting food utilization and weight gain. Compared with the negative control group in the same period, the food intake of the female and male high-dose groups fed with selenium-containing feed decreased significantly in the first week. This is mainly due to the toxic effects of ingesting high doses of selenium[1], as mentioned in the introduction section gastrointestinal toxicity and hepatotoxicity of excess selenium, causing toxic symptoms such as abdominal pain, nausea, Vomiting, etc., which may suppress the appetite of Rats.

The toxic effects of excess selenium can also lead to nutritional imbalances. Although selenium is an essential trace element, higher-than-ideal selenium intake may lead to imbalances in other nutrients. This may interfere with the absorption and utilization of other important nutrients in the Rats, resulting in decreased food intake. For example, there may be interaction and competition between selenium and other trace elements such as copper, iron and zinc. High doses of selenium intake may interfere with the balance of these trace elements, leading to disturbances in their absorption and utilization, leading to deficiency or excess of the corresponding trace elements[9]. In addition, high doses of selenium can interfere with the structure and function of proteins, including the reaction with thiol groups, which may affect the normal structure and function of proteins related to nutrients[9]. Oxidative stress caused by excess selenium should also be considered[10]. Selenium participates in antioxidant responses in moderate amounts but may cause oxidative stress in excess， this may lead to the accumulation of oxidized substances in cells, damage the structure of cell membranes and organelles, and affect the normal transport and metabolism of nutrients.

5. Conclusion

Low and medium doses of selenium have no significant effect on the growth and development of rats, but high doses of feed can affect their food intake, food utilization and weight gain. Therefore, it can be seen that high doses of selenium will bring a series of negative effects, which are not suitable for normal selenium supplementation. Healthy supplementation of selenium is food tonic at low and medium doses. It is suggested that the related selenium supplements is necessary when some diseases related to selenium habits occurred such as Keshan and Kashin-Beck mentioned before.

References

1. Genchi G, Lauria G, Catalano A, Sinicropi MS, Carocci A. Biological activity of selenium and its impact on human health. International Journal of Molecular Sciences. 2023;24(3):2633.
2. Tangjaidee P, Swedlund P, Xiang J, Yin H, Quek SY. Selenium-enriched plant foods: Selenium accumulation, speciation, and health functionality. Frontiers in Nutrition. 2023;9:962312.
3. Sun Y, Wang Z, Gong P, Yao W, Ba Q, Wang H. Review on the health-promoting effect of adequate selenium status. Frontiers in Nutrition. 2023;10:1136458.
4. Ma L, Shen H, Shang X, Zhou S, Lyu B, Zhao X, et al. Dietary Intake of Multiple Nutrient Elements and Associated Health Effects in the Chinese General Population from a Total Diet Study. Nutrients. 2023;15(11):2613.
5. Zhang H, Qiu H, Wang S, Zhang Y. Association of habitually low intake of dietary selenium with new-onset stroke: A retrospective cohort study (2004–2015 China Health and Nutrition Survey). Frontiers in Public Health. 2023;10:1115908.
6. on Nutrition EP, Foods N, NDA FA, Turck D, Bohn T, Castenmiller J, et al. Scientific opinion on the tolerable upper intake level for selenium. EFSA Journal. 2023;21(1).
7. Kunimatsu T, Yamada T, Miyata K, Yabushita S, Seki T, Okuno Y, et al. Evaluation for reliability and feasibility of the draft protocol for the enhanced rat 28-day subacute study (OECD Guideline 407) using androgen antagonist flutamide. Toxicology. 2004;200(1):77-89.

8. Hadrup N, Ravn-Haren G. Toxicity of repeated oral intake of organic selenium, inorganic selenium, and selenium nanoparticles: a review. Journal of Trace Elements in Medicine and Biology. 2023:127235.
9. Ojeda ML, Nogales F, Carrasco López JA, Gallego-López MdC, Carreras O, Alcudia A, et al. Microbiota-Liver-Bile Salts Axis, a Novel Mechanism Involved in the Contrasting Effects of Sodium Selenite and Selenium-Nanoparticle Supplementation on Adipose Tissue Development in Adolescent Rats. Antioxidants. 2023;12(5):1123.
10. Brenneisen P, Steinbrenner H, Sies H. Selenium, oxidative stress, and health aspects. Molecular aspects of medicine. 2005;26(4-5):256-67.
11. The National Health and Family Planning Commission of the People's Republic of China. National Food Safety Standard 28 day Oral Toxicity Test. 2014