The influence of sodium selenite and selenium nanoparticles on the antioxidant status of laboratory rats

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Abstract: The aim of the experiment was to compare the influence of different forms of selenium (sodium selenite, selenium nanoparticles) on the organism of laboratory rats. The males of Wistar albino rat strain were sorted into 3 groups. The first group (n = 5) served as control with no selenium (Se) addition. The second group was fed with mixture containing 1.2 mg/kg of diet of sodium selenite (Na_2SeO_3). The third group (n = 5) was fed with mixture containing selenium nanoparticles (1.2 mg Se/kg of diet). After 30 days of experiment, the rats were slaughtered and antioxidant activity by TEAC and DPPH method were measured in liver, blood and kidney. Oxidative stress of organism was evaluated by levels of superoxide dismutase (SOD) and malonyldialdehyde (MDA) concentration. Statistically significant differences were measured in liver samples by the TEAC method (decrease in Na_2SeO_3 group by 47%, $p < 0.05$) and DPPH (decrease in both selenium groups, Na_2SeO_3 by 43% and SeNPs by 41%, $p < 0.05$). The addition of selenium almost did not affect the concentration of SOD in the organism. There was a small decrease in the level of MDA in the liver and kidney compared to the control group. Results showed selenium nanoparticles may be a potential candidate for further evaluation as selenium supplement with antioxidant properties and be used against selenium deficiency in organism.

Key Words: rats, nanoparticles, TEAC, DPPH, superoxide dismutase, malonyldialdehyde

INTRODUCTION

Selenium is a component of several major metabolic pathways, including antioxidant defense system, reproduction, thyroid hormone metabolism, immune function, and protecting the cells from the harmful effects of free radicals, and viral inhibition (Brown and Arthur 2001, El-Demerdash and Nasr 2014, Hoffmann and Berry 2008). Selenium is important for biosynthesis of selenoenzymes and selenoproteins (Dogan et al. 2016). Selenium concentration in plants is dependent from its content in the soil. In European Union the Se content is relatively low, therefore it is necessary to supplement it into the diet of farm animal (Horky et al. 2016). Nowadays, many researches try to develop new forms of selenium serving as an alternative source for selenium supplement for the animal body. Selenium deficiency leads to a higher rate of lipid peroxidation, damage of membrane structures, impaired reproduction, rapid aging of the organism (Horky et al. 2016). Therefore, the selenium supplement is recommended to the diet not only for humans but also for livestock. However, these Se supplements, especially the inorganic ones, are usually toxic when taken above their nutritional dosage (Zhang and Spallholz 2009).

Recent studies have shown that selenium nanoparticles exhibit lower toxicity by increasing the activity of seleno-enzymes compared to other selenium compounds (selenium, 3,5-selenomethionine, Se-yeast and methylselenocysteine) (Zhang et al. 2007). Moreover, they are able to inhibit the growth of microorganisms and exhibit anticancer activity (Tran and Webster 2011). Nanoparticles can be variously modified and becoming active and thus be the ideal medium for the targeted drug delivery as well as for nutrients in the body (Bai et al. 2017).

MATERIAL AND METHODS

Animals

The feeding experiment was carried out in the experimental facility of Department of Animal Nutrition and Forage Production of Mendel University in Brno. Throughout the whole experiment, microclimatic conditions were observed and controlled at temperature of 23 ± 1 °C and constant humidity of 60%. The photoperiod was maintained at 12 hours of light and 12 hours of darkness with a maximum illumination of 200 lx. As a model animals for this experiment males of the Wistar albino rat were selected and divided into 3 groups of 5 pieces. The average weight of rats at the beginning of the experiment was 139 grams. The first group served as a control with no addition of selenium in their feed. The second group was supplemented with selenium in the form of Na_2SeO_3 at a dose of 1.2 mg/kg of diet. The third group was fed with selenium in form of nanoparticles at a dose of 1.2 mg/kg of diet. All groups were fed with monodiet containing 0.03 mg Se/kg of diet. The experiment duration was 30 days. The animals had access to feed and drinking water ad libitum. At the end of the experiment, the animals were slaughtered (in accordance with the act on the protection of animals against cruelty No. 246/1992 Coll.) and samples of blood, liver and kidney tissue were collected and subjected to biochemical analyses.

Preparation of samples

Liver, kidney: 2 grams of samples from each variant were homogenized with the addition of liquid nitrogen and 1.5 mL of MilliQ water. After homogenization, each sample was sonicated using an ultrasound needle for 2 minutes, shaken for 10 minutes, and centrifuged for 20 minutes at 16 400 rpm and at 4 °C. 100 µL of supernatant was taken from each sample and mixed with 100 µL of 10% trifluoroacetic acid and centrifuged again for 20 minutes at 16 400 rpm and 4 °C. After the centrifugation, the supernatant was taken and analysed.

Blood: 200 µL of sample from each variant were placed into liquid nitrogen for 2 minutes and 500 µL of water was added. Each sample was sonicated with an ultrasound needle for 2 minutes, shaken for 1 minute, and centrifuged for 20 minutes at 16 400 rpm and at 4 °C. 200 µL of supernatant was taken from each sample and mixed with 200 µL of 10% trifluoroacetic acid. The samples were again centrifuged for 20 minutes at 16 400 rpm and 4 °C. After centrifugation, the supernatant was analysed.

Preparation of selenium nanoparticles modified by glucose and reduced by cysteine

250 mg of glucose was dissolved in Milli Q water (40 mL) with constant stirring. Subsequently, 5 mL of Na_2SeO_3. $5H_2O$ (0.263 g/50 mL) were added and the pH adjusted to 6.3 with 1 M HCl dropwise. Subsequently, 1 mL of cysteine (1.21/50 mL) was added. The mixture was decolorized to orange and the pH increased to 7.3.

Oxidative status determination

For determination of antioxidant activity, a BS-400 automated spectrophotometer (Mindray, China) was used. It was composed of cuvette space tempered to 37 ± 1 °C, reagent space with a carousel for reagents (tempered to 4 ± 1 °C), sample space with a carousel for preparation of samples and an optical detector. Transfer of samples and reagents is provided by robotic arm equipped with a dosing needle (error of dosage up to 5% of volume). Cuvette contents were mixed by an automatic mixer including a stirrer immediately after addition of reagents or samples. Contamination was reduced due to its rinsing system, including rinsing of the dosing needle as well as the stirrer by MilliQ water.

TEAC

Briefly, ABTS• (54.9 mg) was dissolved in 20 mL of phosphate buffer (pH 7.0; 5 mM) and activated to cation of ABTS+ radical by addition of MnO_2 (1 g) under occasional stirring for 30 min. Subsequently, volume of 15 µL of sample was added. Solution was subsequently diluted by phosphate buffer to absorbance (t0) 0.500 ± 0.01. Absorbance of solution was measured at $\lambda = 734$ nm.

DPPH

150 µL of R1 reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl - DPPH) was pipetted into plastic cuvette. Subsequently, volume of 15 µL of sample was added. This method is based on the ability of stable free radical of 2,2-diphenyl-1-picrylhydrazyle to react with donors of hydrogen. DPPH has

strong absorption in UV-VIS spectrum, absorbance was measured for 12 min at λ = 505 nm. To assess the production of free radicals absorbance difference of the reagent without sample and reagent with sample after ten-minute incubation was taken.

Photometric determination of malondialdehyde according to Hong (2000)

The principle of malondialdehyde determination is reaction between malondialdehyde (MDA) with thiobarbituric acid (TBA) under formation of TBA-MDA-TBA adduct that absorbs strongly at 535 nm. Trichloroacetic acid (TCA) is added to the sample because of its ability to precipitate proteins, bilirubin, unsaturated fatty acids and lipoproteins. A 300 µL sample of blood plasma was mixed with 10 µL 0.5 M solution of butylated hydroxytoluene (BHT) in 96% ethanol (*v*/v) and 310 µL 20% TCA (*v*/*v*) prepared in 0.6 M HCl. After 20 min incubation on ice mixture was centrifuged at 11.000 rpm. for 15 min. Subsequently, 400 µL of supernatant was mixed with 800 µL of 30 mM TBA and mixture was incubated in a thermomixer Comfort (Eppendorf, Germany) at 90 °C for 30 min. After cooling in ice MDA absorbance was measured using a spectrophotometer at 535 nm and the concentration was subtracted from the calibration curve.

Determination of superoxide dismutase (SOD)

Kit 19160 SOD (Sigma Aldrich, USA) was used for assay of superoxide dismutase (SOD, EC 1.15.1.1.). A 200 µL volume of reagent R1 (WTS solution diluted 20 times with buffer) was pipetted into a plastic cuvette and agent was incubated at 37 °C for 108 s (1 min, 48 s). Afterwards, a 20 µL volume of sample was pipetted and in 378 s (6 min, 18 s), the reaction was started by adding a 20 µL volume of reagent R2 (enzyme solution 167 times diluted with buffer). Mixture was incubated for 72 s (1 min, 12 s) and then absorbance was measured at λ = 450 nm. Kinetic reaction was measured for 108 s (3 min) and absorbance was read every 9 s.

Statistics

The data were processed statistically using STATISTICA.CZ, version 12.0 (the Czech Republic). The results were expressed as mean ± standard deviation (SD). Statistical significance was determined using ANOVA and Scheffé's test (one-way analysis).

RESULTS AND DISCUSSION

The aim of the experiment was to determine whether new form of selenium, based on nanotechnology, can influenced the antioxidant status of rats. For this purpose, TEAC and DPPH method was applied to determination of antioxidant activity. Further, activity of SOD was determined because it is an important endogenous antioxidant enzyme that acts as a component of the first line of defence system against reactive oxygen species (ROS) (Ighodaro and Akinloye 2017). And as marker of lipid peroxidation and oxidative stress concentration of MDA was used.

Antioxidant activity was determined using TEAC and DPPH assays. Trolox was used as the standard. From the results is obvious that antioxidant activity of blood reached the similar levels in selenium treated groups (Na_2SeO_3, SeNPs) and control group (Figure 1). This effect could be explained by the ability of blood to quickly cope with oxidative stress (Horký 2014). Similar results have been confirmed by other authors (Urbankova et al. 2018, Horky et al. 2016). In the case of antioxidant status of liver, the significant difference was estimated between Na_2SeO_3 (decrease by 47%, $p < 0.05$) treated rats and control measured by TEAC method (Figure 1A). And also between Na_2SeO_3 (by 43%) and SeNPs (by 41%) group compared with control measured by DPPH method (Figure 1B). But not between groups fed by Na_2SeO_3 and SeNPs. The TEAC and DPPH method showed reduced antioxidant status of the experimental group in comparison to the control groups. In kidney samples in selenium groups decrease was measured but with no significant difference

Selenium supplementation (both Na_2SeO_3 and selenium nanoparticles) did not influence the enzymatic activity of SOD in blood, kidney and liver compared to the control sample (Figure 2A). It is obvious that Se supplementation (both of Na_2SeO_3 and SeNPs) did not reduce the activity of the important antioxidant defense against oxygen radicals in rats.

The disfunctions of the antioxidant system may be expressed by an increased degree of lipid peroxidation. In this study the lipid peroxidation level measured by (MDA) content was not significantly

different in experimental groups compared with control in all samples (liver, blood, kidney). Slight decrease was in blood, liver and kidney samples in SeNPs group (Figure 2B). Also López et al. (2010) observed lower levels of MDA when selenium (0.4 mg of Se/kg of diet) was added to the diet.

Figure 1 Influence of sodium selenite and selenium nanoparticles on antioxidant activity measured by TEAC (A) and DPPH (B) method

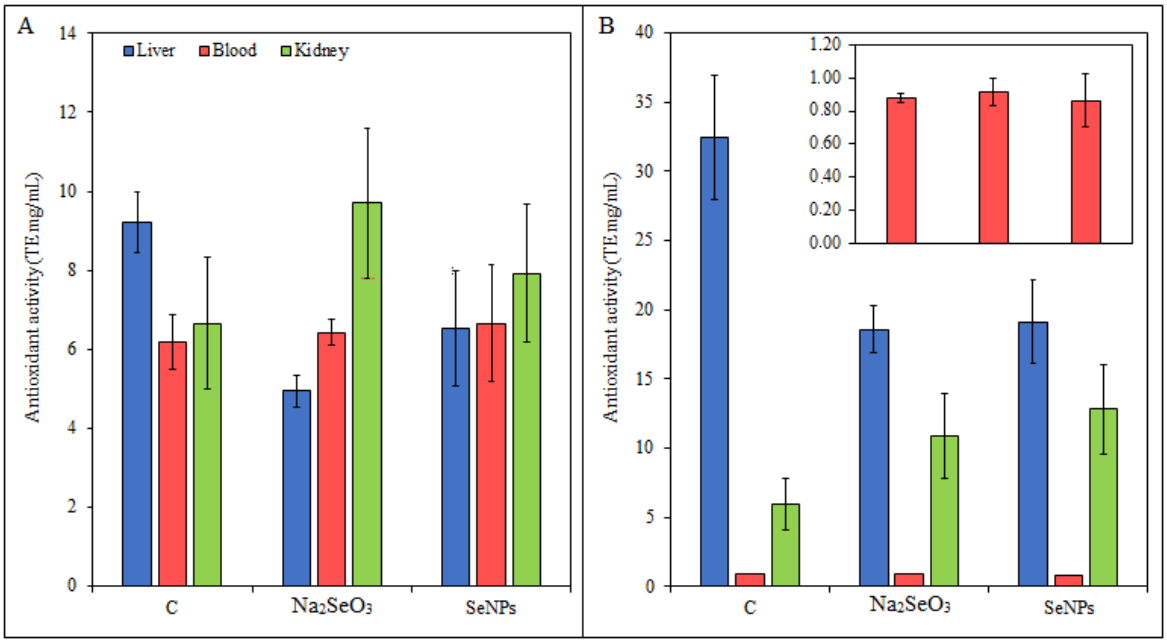
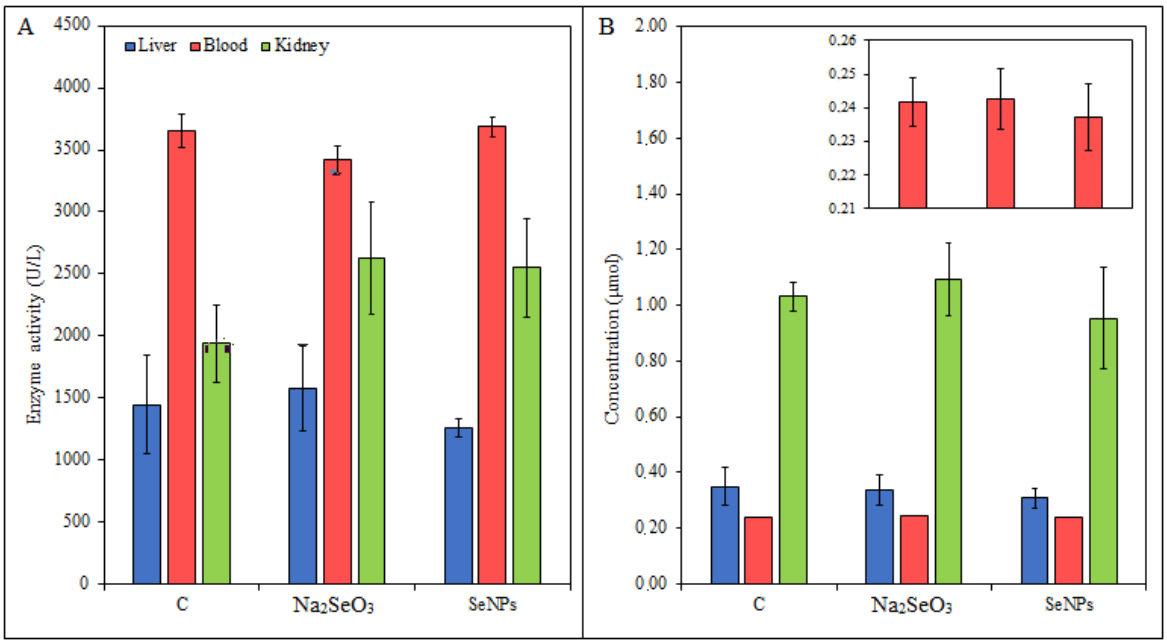


Figure 2 Influence of sodium selenite and selenium nanoparticles on SOD activity (A) and MDA concentration (B)



CONCLUSION

The Na_2SeO_3 and SeNPs supplementation of rats did not cause the damage the antioxidant system. The levels of the main indicators of oxidative stress were in the standard. Research suggests that

selenium nanoparticles can be used in nutrition of monogastric animals. For further investigation the selenium nanoparticles are perspective option in solving the issue of selenium supplementation.

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