Antioxidant status of rats' blood and liver affected by sodium selenite and selenium nanoparticles

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Background. Selenium is an essential element; however, at higher doses, it can be toxic. Therefore, alternative nanotechnological solutions are required to overcome toxicological issues, rather than conventional alternatives. Nanoparticles show new and promising properties that may be able to suppress toxicity while maintaining the positive effects of selenium on an organism. The aim of the experiment was to determine the influence of sodium selenite and selenium nanoparticles on the antioxidant status of rats.

Methods. The males of the outbreed rat strain Wistar albino were selected as a model organism. Animals were fed different forms of selenium. The control group was given a mixture without selenium addition, whereas other groups were fed a mixture containing sodium selenite, Se-49, and Se-100 selenium nanoparticles, respectively. The duration of the trial was 30 days.

Results. Analysis of blood and liver was performed where the concentration of reduced (GSH) and oxidised (GSSG) glutathione, and total selenium content were measured. In the liver, a significant reduction in GSSG was found for all experiment groups. Blood samples showed a significant reduction in GSH and an increase in GSSG.

Discussion. These results show that selenium nanoparticles may be an alternative to dietary selenium for animal organisms.

1   **Antioxidant status of rats' blood and liver affected by sodium selenite and**

2   **selenium nanoparticles**

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19   **Abstract**

20   **Background.** Selenium is an essential element; however, at higher doses, it can be toxic.

21   Therefore, alternative nanotechnological solutions are required to overcome toxicological issues,

22   rather than conventional alternatives. Nanoparticles show new and promising properties that may

23   be able to supress toxicity while maintaining the positive effects of selenium on an organism. The

24   aim of the experiment was to determine the influence of sodium selenite and selenium

25   nanoparticles on the antioxidant status of rats.

26   **Methods.** The males of the outbreed rat strain Wistar albino were selected as a model organism.

27   Animals were fed different forms of selenium. The control group was given a mixture without

28   selenium addition, whereas other groups were fed a mixture containing sodium selenite, Se-49,

29   and Se-100 selenium nanoparticles, respectively. The duration of the trial was 30 days.

30   **Results.** Analysis of blood and liver was performed where the concentration of reduced (GSH)

31   and oxidised (GSSG) glutathione, and total selenium content were measured. In the liver, a

32   significant reduction in GSSG was found for all experiment groups. Blood samples showed a

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34   **Discussion.** These results show that selenium nanoparticles may be an alternative to dietary

35   selenium for animal organisms.

36

Introduction

37

38   The antioxidant status of animals can be positively affected by the addition of antioxidants,

39   including vitamin E and antioxidant enzyme cofactors such as selenium, which is an important

40   element in selenoproteins, of which at least 16 have an antioxidant role. Interaction between Se

41   and Vitamin E may increase the production of glutathione peroxidase, which is an important aspect

42   of the antioxidant system (Arruda et al. 2015; Horky et al. 2016b; Chen et al. 2016a; Chen et al.

43   2016b; Skalickova et al. 2017; Tran & Webster 2011; Wang et al. 2007; Zhang et al. 2001).

44   Moreover, selenium supports immune response, where in enzyme deiodinase it is necessary for

45   conversion of thyroxine (T_4) to more active triiodothyronine (T_3) (Bunglavan et al. 2014).

46

47   The selenium content in soils in Europe is generally low and as such, it should be added to livestock

48   feed (Horky et al. 2012; Kursa et al. 2010). The two most widely used inorganic selenium forms

49   are selenate and selenite. Both can be converted into less toxic insoluble elemental selenium forms.

50   However, the biological nature of this reaction is not yet known (Chen et al. 2016b). In organic

51   form, selenium is used as a component of amino acids (e.g., selenomethionine) (Horky et al. 2013;

52   Mohapatra et al. 2014). Selenium in a low dose is an essential element important in several

53   physiological processes such as synthesis of selenocysteine, coenzyme Q, glutathione peroxidase,

54   and thioredoxin reductase. At higher doses, selenium may be toxic (Fernandez-Llamosas et al.

55   2016; Horky 2014).

56

57   Accordingly, alternative nanotechnological solutions are required, as opposed to conventional

58   alternatives. In this context , nanoparticles indicate new and promising properties that can

59   potentially supress toxicity, while maintaining the positive effects of selenium on an organism

60 (Arruda et al. 2015; Fernandez-Llamosas et al. 2016; Horky et al. 2012; Mohapatra et al. 2014;

61 Skalickova et al. 2017). The synthesis and application of selenium nanoparticles (SeNPs) has

62 gained increased attention due to the number of benefits it presents, such as low toxicity,

63 biocompatibility, and chemical stability (Zhang et al. 2001). Nowadays, selenium nanoparticles

64 are widely used as a nutritional supplement (Wang et al. 2007). Selenium nanoparticles have been

65 found to show lower cytotoxicity compared to inorganic selenium compounds, and have excellent

66 anti-cancer and therapeutic properties (Anjum et al. 2016). Zhang et al. (2008) showed that

67 selenium nanoparticles exhibited novel in vitro and in vivo antioxidant activities using the

68 activation of selenoenzymes. On the other hand, the antiproliferative activity of these nanoparticles

69 according to an unknown mechanism have also been identified (Peng et al. 2007), as well as their

70 antimicrobial effects (Hegerova et al. 2017; Tran & Webster 2011).

71 The aim of our study was to compare two different forms of dietary nanoselenium with sodium

72 selenite to show whether selenium nanoparticles can increase the antioxidant status of rat

73 metabolism, and serve as an alternative source of nutritional supplement for an animal organism.

74

75 **Materials and methods**

76 *Animals*

77 The experiments were performed with the approval of the Ethics Commission at the Faculty of

78 AgriSciences, Mendel University in Brno, Czech Republic (project number 02154869). The

79 experiment was carried out in the experimental facility of the Department of Animal Nutrition and

80 Forage Production of Mendel University in Brno, in accordance with the Act on the protection of

81 animals against cruelty No. 246/1992 Coll. Throughout the entire experiment, microclimatic

82 conditions were measured and controlled at $23 \pm 1°C$, and at a constant humidity of 60%. The light

83   regime was maintained at 12 h of light and 12 h of darkness, with a maximum illumination of 200

84   lx.

85   Laboratory male rats of the outbreed strain Wistar albino were selected as model animals, and

86   included 32 rats aged 28 days, with an average initial weight of 150 ± 5 g. The rats were divided

87   into four groups of eight rats each. The first group served as a control with no addition of selenium

88   to their feed. The second group was supplemented with selenium in the form of Na_2SeO_3 at a dose

89   of 1.2 mg/kg/diet. Group three and four were fed with selenium in the form of Se-49 and Se-100

90   nanoparticles at a dose of 1.2 mg/kg/diet, respectively. Groups two, three, and four were fed

91   monodietus containing 0.03 mg Se/kg/diet. The experiment duration was 30 days. The animals

92   had access to feed and drinking water *ad libitum*. At the end of the experiment, the animals were

93   putted to death and blood and liver samples were collected and subjected to chemical analyses.

94

95   *Chemicals and instruments*

96   Methanol, trifluoroacetic acid (TFA), sodium selenite, Poly(vinyl alcohol) (PVA 49 kDa or PVA

97   100 kDa), reduced glutathione (GSH), and oxidised glutathione (GSSG) were obtained from

98   Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Deionised water

99   underwent demineralisation by reverse osmosis using the instrument Aqua Osmotic 02

100 (AquaOsmotic, Tisnov, Czech Republic), and was subsequently purified using Millipore RG

101 (Millipore Corp., USA, 18 MΩ) to gain MilliQ water. The average particle size distribution was

102 determined by quasi-elastic laser light scattering using a Malvern Zetasizer (NANO-ZS, Malvern

103 Instruments Ltd., Worcestershire, United Kingdom). Solutions of nanoparticles were measured

104 according to experimental conditions stated in (Dostalova et al. 2016). The structures of

105 nanoparticles were observed using scanning electron microscopy (FE Tescan Mira II LMU, Brno,

106 Czech Republic) under the conditions employed in (Dostalova et al. 2016; Chudobova et al. 2014).

107 Characterisation of nanoparticles is shown in Fig. 1.

108

109 *Preparation of selenium nanoparticles*

110 Se-49

111 PVA 49 kDa (0.19 g) was added to a solution of 1.88 mL $Na_2SeO_3 \cdot 5H_2O$ (2.63 g/50 mL) in water

112 (80 mL). Cysteine (9 mg/mL) was added with mixing, and left for 2 h. At this stage, the colour

113 turned a light orange and water was added to achieve a final 100 mL volume.

114 Se-100

115 Preparation was the same as in the previous instance, with the exception of using PVA 100 kDa

116 instead of PVA 49 kDa. Undissolved PVA was filtered off. After the addition of cysteine, the

117 colour turned to orange and water was added to gain a final 100 mL volume.

118

119 *Preparation of samples for GSH and GSSG detection*

120 Liver: 2 g of samples from each variant were homogenised in a fritted bowl with the addition of

121 liquid nitrogen and 1.5 mL water. Following homogenisation, each sample was sonicated using an

122 ultrasound needle for 2 min, shaken for 10 min, and centrifuged for 20 min at 25 000 g, and at

123 4°C. Following on, 100 μL of supernatant was taken from each sample and mixed with 100 μL of

124 10% TFA, and centrifuged again for 20 min at 25 000 g and 4°C. Following centrifugation, the

125 supernatant was taken and analysed by HPLC-ED (Fig. 2).

126 Blood: sample processing was performed by pipetting 200 μL of sample from each variant, placing

127 it into liquid nitrogen for 2 min and adding 500 μL water. Each sample was sonicated with an

128 ultrasound needle for 2 min, shaken for 1 min, and centrifuged for 20 min at 25 000 g and at 4°C.

129   Then, 200 μL of supernatant was taken from each sample and mixed with 200 μL of 10% TFA.

130   The samples were again centrifuged for 20 min at 25 000 g and 4°C. Following centrifugation, the

131   supernatant was analysed by HPLC-ED (Fig. 2).

132

133

134   *Preparation of samples for selenium detection*

135   Samples of liver weighing 0.3 g and samples of blood weighing 0.5 g were disintegrated by dry

136   method in a muffle furnace (LAC, Czech Republic) and mineralised in 2.5 mL concentrated nitric

137   acid (Horky et al. 2016a). The preparation scheme is shown in Fig. 2.

138

139   *Determination of reduced and oxidised glutathione and selenium*

140   Reduced and oxidised glutathiones were determined using high performance liquid

141   chromatography with electrochemical detection (HPLC-ED). Experimental conditions were

142   adopted from (Zitka et al. 2012). Selenium was determined using a 280Z Agilent Technologies

143   atomic absorption spectrometer (Agilent, USA) with electrothermal atomisation under the

144   conditions stated in (Horky et al. 2016b).

145

146   *Statistics*

147   The data were processed statistically using Statistica.Cz, version 10.0 (Czech Republic). The Three

148   measurements were taken, $P < 0.05$ was considered significant using ANOVA, and Scheffe's

149   method was used determining parameters GSH, GSSG, and Se.

150

151   **Results**

152   In the experiment, conventional (sodium selenite) and alternative (selenium nanoparticles) forms

153   of selenium as sources of this element for animal organisms were investigated. Oxidative

154   glutathione, oxidised glutathione, and selenium in blood and liver were selected as markers of

155   oxidative stress. The level of oxidised and reduced glutathione showed a smaller increase in liver

156   and blood, with the exception of GSH in liver samples. In the liver tissue, a significant decrease

157   of 30% was found in the Na_2SeO_3 group (P <0.05), together with both groups containing selenium

158   nanoparticles, Se-49 at 34% (P <0.05), and Se-100 at 29% (P <0.05) (Fig. 3A). In blood, a

159   statistically significant reduction in GSH was found for all control groups (Na_2SeO_3 by 72%, Se-

160   49 by 59%, Se-100 by 67%; P <0.05). Conversely, a 17% increase in GSSG was found in the

161   Na_2SeO_3 group (P <0.05). In the case of nanoparticle treated experimental groups, Se-49 increased

162   by 51% (P <0.05), and Se-100 by 47% (P <0.05) (Fig. 3B).

163   Additionally, we also determined selenium content. In liver samples, a significant increase of 85%

164   was observed in selenium concentration in the Na_2SeO_3 group (P <0.05), an increase in Se-49 by

165   30% (P <0.05), and in Se-100 by 73% (P <0.05), compared to rats in the control group (Fig. 4A).

166   The level of selenium in blood was also the highest in the Na_2SeO_3 group, where there was an

167   increase of 240% (P <0.05) against the control group. Other groups showed significant increase as

168   well: Se-49 by 18% (P <0.05) and Se-100 by 64% (P <0.05) (Fig. 4B).

169

170   **Discussion**

171   In our experiment, the effect of an alternative source of selenium, i.e. selenium nanoparticles, was

172   studied in terms of influencing the antioxidant potential of a rat organism. Antioxidant activity is

173   an indicator of the ability of the entire body and selected organs to defend against free radicals.

174   Reducing the antioxidant activity of the organism leads to an intensification of oxidative stress that

175 affects the entire body, increases the risk of injury, reduces performance, and deteriorates certain

176 diseases.

177 At present, there is relatively little existing work on the use of nanoselenium in diet. In a study on

178 the reduction of radioactive gamma radiation, selenium particles were given at a dose of 20 mg

179 Se/kg of body weight per day (i.e. 3 mg Se/animal/day), and 0.1 mg Se/kg of body weight per day

180 (0.015 mg Se/animal/day). The level of selenium and GSH was not affected (El-Batal et al. 2012).

181 In contrast, the selenium level was increased by 64% in the Se-100 group and GSH level decreased

182 in all our experimental groups in blood samples. However, it should be noted that the animals had

183 not been exposed to gamma rays, which will undoubtedly affect animals' antioxidant status.

184 The effect of selenium nanoparticles applied to a sugar carrier (glucose) was studied (Horky et al.

185 2016a). Selenium particles were given at a dose of 0.02 mg Se/animal/day. After 10 days, an

186 increase in GSH and total GPx activity in blood was found, which is inconsistent with our trial, in

187 which GSH elevation did not occur. In another experiment on rats (Hadrup et al. 2016), the effect

188 of selenium and selenium nanocomponents' addition (0.05 mg/kg bw and 0.5 mg/kg bw) was

189 compared with a control group. The doses were added to feed as solutions using a gastric tube

190 every other day, and urine samples were collected. After 14 days, no toxic effects and no evidence

191 of weight reduction were observed, compared to the control.

192 In the past, rat experiments were conducted to compare the effect of organic and inorganic

193 selenium. According to (Sochor et al. 2012), the addition of 1.5 mg of Se in organic form (yeast)

194 increased GSH and GPx activity, when compared to sodium selenite. It follows from the results

195 found that the addition of 1.5 mg may increase the antioxidant potential of animals, without the

196 occurrence of signs of toxicity. Another group of authors (Kominkova et al. 2015) indicate the

197 optimal amount of GSH and GSSG as 90% or 10%, respectively. In our experiment, higher levels

198   of GSSG (oxidised form) were observed in all selenium addition groups. In blood, the difference

199   was the most significant. It is possible that our selected amount and form of selenium (1.2 mg/kg

200   diet) already had depression in the optimal GSH:GSSG ratio. However, our results correspond to

201   results in a study (Blahova et al. 2014), where measured concentrations in the liver ranged from 6

202   to 800 nmol/g for GSH, and from 30 to 800 nmol/g for GSSG. Similar results for the liver were

203   also recorded in a study (Guan et al. 2003). For blood samples, we achieved a higher concentration

204   of GSH and GSSG than in (Guan et al. 2003; Horky et al. 2016a); this difference was most likely

205   caused by another approach to sample preparation and the analysis itself.

206

207   **Conclusions**

208   The experiment investigated the effect of selenium nanoparticles on the antioxidant status of

209   laboratory rats. Alterations in reduced and oxidised glutathiones revealed marked changes in

210   antioxidant status-based selenium treatment; however, we confirmed that the nano-form of

211   selenium has fewer negative effects than the standard form. This leads us to support the idea of

212   using nanoSe as an alternative source of selenium. Moreover, the possibilities of various

213   modifications of the surface of particles is an additional advantage of using these particles, rather

214   than standard inorganic forms, as we show that such nanoSe can be employed without harming

215   animals. It will be appropriate to test these selenium sources at even lower concentrations in order

216   to avoid potential toxicity.

217

218 **Captions for Figures**

219 **Figure 1**

220 (A) Hydrodynamic diameter distribution of nanoselenium particles Se-49 measured by quasi-

221 elastic laser light scattering with a Malvern Zetasizer. Inset (a) shows the SEM image of Se-49

222 obtained from FE Tescan Mira II LMU. (B) Hydrodynamic diameter distribution of nanoselenium

223 particles Se-100. Inset (b) shows SEM image of Se-100.

224

225 **Figure 2**

226 Workflow diagram of the experiment. (A) Tissue extraction and blood collecting; (B) liver and

227 blood; (C) tissue and/or blood microwave assisted mineralisation; (D) determination of Se content

228 by AAS and GSH, GSSG content by HPLC-ED. Photography was provided by Zuzana Lackova.

229

230 **Figure 3**

231 Influence of different forms of selenium on the level of GSH and GSSH in (A) liver and (B) blood.

232

233 **Figure 4**

234 Effect of different forms of selenium on the concentration of selenium in (A) liver and (B) blood.

235

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332

Figure 1

Characterization of nanoparticles

(A) Hydrodynamic diameter distribution of nanoselenium particles Se-49 measured by quasi-elastic laser light scattering with a Malvern Zetasizer. Inset (a) shows SEM image of Se-49 obtained on FE Tescan Mira II LMU. (B) Hydrodynamic diameter distribution of nanoselenium particles Se-100. Inset (b) shows SEM image of Se-100.
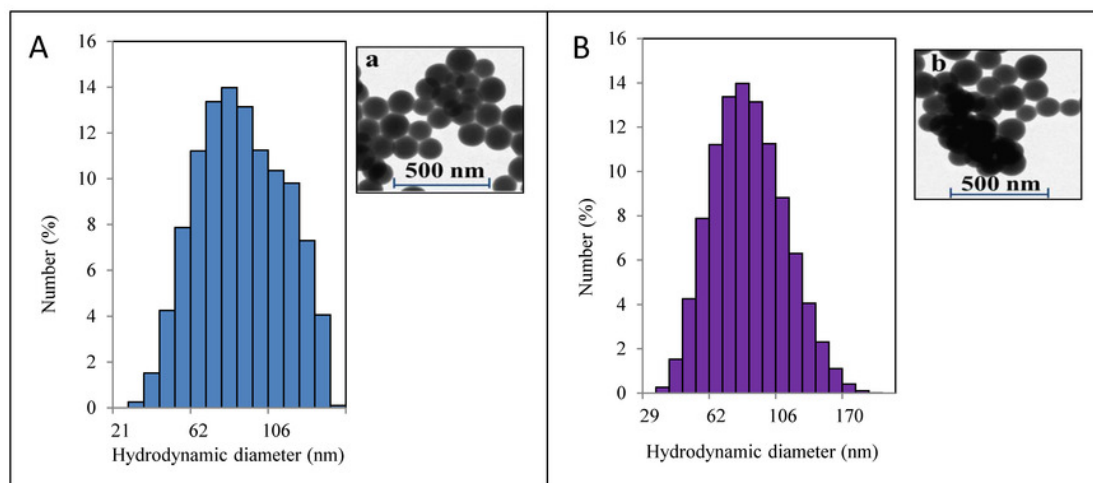
Figure 2

Sample preparation

Workflow diagram of the experiment. (A) Tissue extraction and blood collecting, (B) Liver and blood, (C) tissue and/or blood microwave assisted mineralization, (D) determination of Se content by AAS and GSH, GSSG content by HPLC-ED. Photos by Zuzana Lackova.
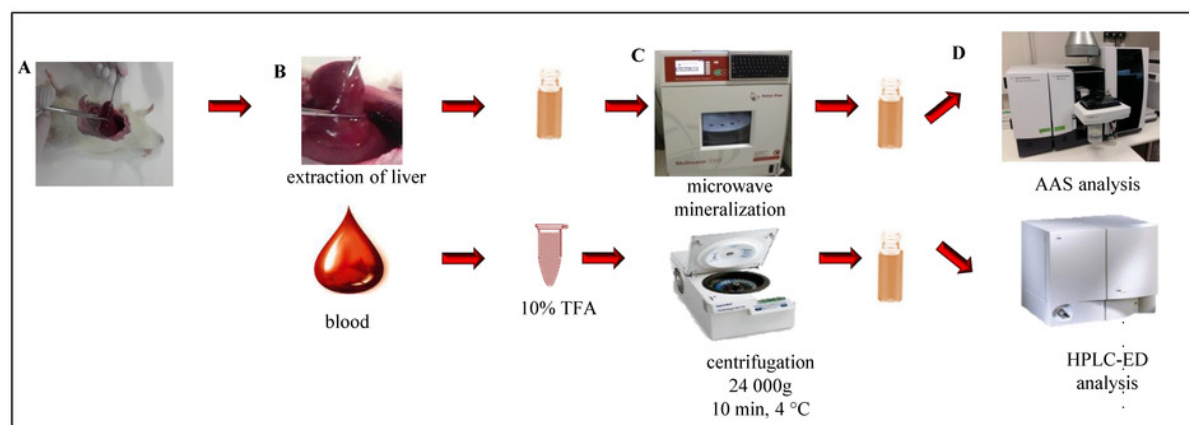
Figure 3

Glutathiones

Influence of different forms of selenium on the level of GSH a GSSH in (A) liver and (B) blood.
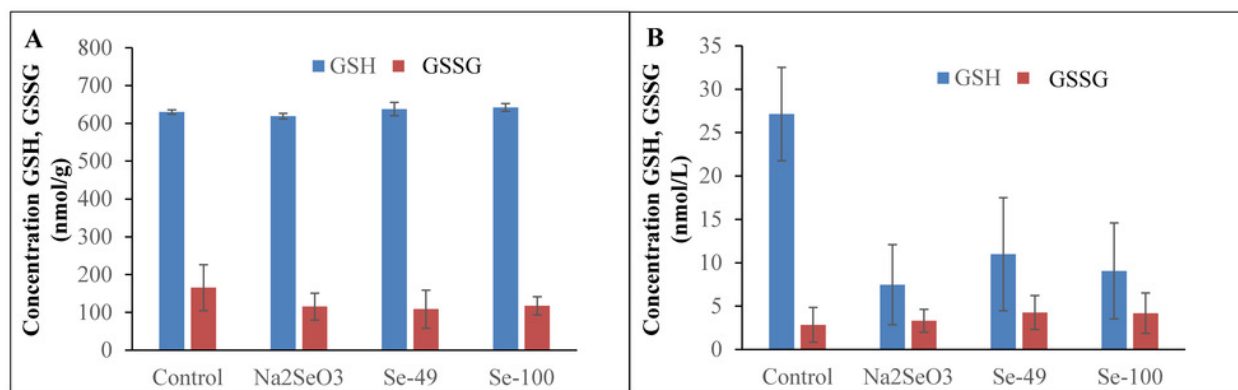
Figure 4

Selenium

Effect of different forms of selenium on concentration of selenium in (A) liver and (B) blood.