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

Lenka Urbankova<sup>1</sup>, Pavel Horky<sup>1</sup>, Jiri Skladanka<sup>1</sup>, Magdalena Pribilova<sup>1</sup>, Vendula Smolikova<sup>1</sup>, Pavel Nevrkla<sup>1</sup>, Natalia Cernei<sup>1</sup>, Zuzana Lackova<sup>1</sup>, Josef Hedbavny<sup>1</sup>, Andrea Ridoskova<sup>1</sup>, Vojtech Adam<sup>1</sup>, Pavel Kopel<sup>Corresp.</sup><sup>1</sup>

<sup>1</sup> Mendel University of Agriculture and Forestry, Brno, Czech Republic

Corresponding Author: Pavel Kopel  
Email address: pavel.kopel@mendelu.cz

**Background.** The aim of the experiment was to determine the influence of sodium selenite and selenium nanoparticles on antioxidant status of rats.

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

**Results.** The analysis of blood and liver was performed where concentration of reduced (GSH) and oxidized (GSSG) glutathione, and the total selenium content were measured. In liver, a significant reduction in GSSG was found in all experimental 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 the animal organism.

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Lenka Urbankova <sup>a</sup>, Pavel Horky <sup>a</sup>, Jiri Skladanka <sup>a</sup>, Magdalena Pribilova <sup>a</sup>, Vendula Smolikova <sup>b</sup>, Pavel Nevrla <sup>c</sup>, Natalia Cernei <sup>b,d</sup>, Zuzana Lackova <sup>b,d</sup>, Josef Hedbavny <sup>b</sup>, Andrea Ridoskova <sup>b</sup>, Vojtech Adam <sup>b,d</sup>, Pavel Kopel <sup>b,d\*</sup>

<sup>a</sup> Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

<sup>b</sup> Department of Chemistry and Biochemistry, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

<sup>c</sup> Department of Animal Breeding, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic

<sup>d</sup> Central European Institute of Technology, Brno University of Technology, Purkynova 123, CZ-612 00 Brno, Czech Republic

\*Corresponding author. Tel.: +420 545 133 350.

*E-mail address:* paulko@centrum.cz

# 16 Abstract

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18 selenium nanoparticles on antioxidant status of rats.

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20 Animals were fed with different forms of selenium. The control group was given mixture without  
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29 **Keywords:** Selenium nanoparticles; glutathione; Antioxidant; rat; Animal nutrition

## 30 Introduction

31 The antioxidant status of animals can be positively affected by addition of antioxidants, including  
32 vitamin E and antioxidant enzyme cofactors, such as selenium, which is an important element in  
33 selenoproteins, of which at least 16 have an antioxidant role. Interaction between Se and Vitamin  
34 E may increase the production of glutathione peroxidase, which is an important part of the  
35 antioxidant system (Arruda et al. 2015; Horky et al. 2016b; Chen et al. 2016a; Skalickova et al.  
36 2017; Tran & Webster 2011; Wang et al. 2007; Zhang et al. 2001). Moreover, selenium supports  
37 immune response, where it is in enzyme deiodinase necessary for conversion of thyroxine ( $T_4$ ) to  
38 more active triiodothyronine ( $T_3$ ) (Bunglavan et al. 2014).

39 The selenium content in soils in Europe is generally low therefore, it should be added to livestock  
40 feed (Horky et al. 2012; Kursa et al. 2010). The two most widely used inorganic selenium forms  
41 are selenate and selenite. Both can be converted into less toxic insoluble elemental selenium  
42 forms. However, the biological nature of this reaction is not known yet (Chen et al. 2016b). In  
43 an organic form, selenium is used as a component of amino acids (e.g., selenomethionine) (Horky  
44 et al. 2013; Mohapatra et al. 2014). Selenium in a low dose is an essential element important in  
45 several physiological processes, such as synthesis of selenocysteine, coenzyme Q, glutathione  
46 peroxidase and thioredoxin reductase. At higher doses, selenium may be toxic (Fernandez-  
47 Llamosas et al. 2016; Horky 2014).

48 Thus, alternative nanotechnological solutions are searched instead of conventional alternatives as  
49 nanoparticles show new promising properties, which could suppress toxicity with maintaining the  
50 positive effects of selenium on an organism (Arruda et al. 2015; Fernandez-Llamosas et al. 2016;  
51 Mohapatra et al. 2014; Skalickova et al. 2017). The synthesis and application of selenium  
52 nanoparticles (SeNPs) attracted increased attention due to several benefits, such as low toxicity,  
53 biocompatibility and chemical stability (Zhang et al. 2001). Nowadays, selenium nanoparticles  
54 are widely used as a nutrition supplement (Wang et al. 2007). Selenium nanoparticles have been

found to show lower cytotoxicity, compared to inorganic selenium compounds, and have excellent anti-cancer and therapeutic properties (Anjum et al. 2016). Zhang et al. showed that selenium nanoparticles exhibited novel *in vitro* and *in vivo* antioxidant activities using the activation of selenoenzymes (Zhang et al. 2008). On the other hand, there have been identified antiproliferative activity of these nanoparticles with unknown mechanism (Peng et al. 2007) as well as their antimicrobial effects (Hegerova et al. 2017; Tran & Webster 2011).

The aim of our study was to compare two different forms of dietary nanoselenium with sodium selenite to show whether selenium nanoparticles can increase the antioxidant status of rat metabolism and serve as an alternative source of nutrition supplements for an animal organism.

## Materials and methods

### *Animals*

The experiments were performed with the approval of the Ethics Commission at the Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic (project Number 02154869). The experiment was carried out in the experimental facility of the Department of Animal Nutrition and Forage Plant of Mendel University in Brno, in accordance with the act on the protection of animals against cruelty No. 246/1992 Coll. Throughout the whole experiment, microclimatic conditions were measured and controlled at  $23 \pm 1$  °C at constant humidity of 60%. The light regime was maintained at 12 h of light and 12 h of dark with a maximum illumination of 200 lx.

Laboratory rats of the outbred strain Wistar albino were selected as model animals in number of 32 pieces with an average initial weight of  $150 \pm 5$  g. The rats were divided into 4 groups of 8 pieces. The first group was a control with no addition of selenium in their feed. The second group was supplemented with selenium in the form of  $\text{Na}_2\text{SeO}_3$  at a dose of 1.2 mg/kg/diet. The third and fourth group were fed with selenium in form of Se-49 and Se-100 nanoparticles at a dose of

1.2 mg/kg/diet, respectively. The groups 2, 3 and 4 were fed with monodietus containing 0.03 mg Se/kg/diet. The experiment duration was 30 days. The animals had an access to feed and drinking water *ad libitum*. At the end of the experiment, the animals were sacrificed and samples of blood and liver were collected and subjected to chemical analyses.

### *Chemicals and instruments*

Methanol, trifluoroacetic acid (TFA), sodium selenite, Poly(vinyl alcohol) (PVA 49 kDa or PVA 100 kDa), reduced glutathione (GSH) and oxidized glutathione (GSSG) were obtained from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Deionised water underwent demineralization by reverse osmosis using the instruments Aqua Osmotic 02 (AquaOsmotic, Tisnov, Czech Republic) and then it was subsequently purified using Millipore RG (Millipore Corp., USA, 18 MΩ) – MilliQ water. The average particle size distribution was determined by quasi-elastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, United Kingdom). Solutions of nanoparticles were measured according to experimental conditions stated in (Dostalova et al. 2016). The structures of nanoparticles were observed using scanning electron microscopy (FE Tescan Mira II LMU, Brno, Czech Republic) under the conditions showed in (Dostalova et al. 2016; Chudobova et al. 2014). Characterization of nanoparticles is given in Fig. 1.

### *Preparation of selenium nanoparticles*

#### *Se-49*

PVA 49 kDa (0.19 g) was added to a solution of 1.88 mL Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O (2.63 g/50 mL) in water (80 mL). Cysteine (9 mg/mL) was added with mixing and left for 2 h. Then, the colour turned to light orange and water was added to final 100 mL volume.

#### *Se-100*

102 The preparation was the same as in previous case with one exception of using PVA 100 kDa  
103 instead of PVA 49 kDa. Undissolved PVA was filtered off. After addition of cysteine, the colour  
104 turned to orange and water was added to final 100 mL volume.

#### 105 *Preparation of samples for GSH and GSSG detection*

106 Liver: Two grams of samples from each variant were homogenized in a fritted bowl with the  
107 addition of liquid nitrogen and 1.5 mL of water. After homogenization, each sample was  
108 sonicated using an ultrasound needle for 2 min, shaken for 10 min, and centrifuged for 20 min at  
109 25,000 g and at 4 °C. 100 µL of supernatant was taken from each sample and mixed with 100 µL  
110 of 10% TFA and centrifuged again for 20 min at 25,000 g and 4 °C. After the centrifugation, the  
111 supernatant was taken and analysed by HPLC-ED (Fig. 2).

112 Blood: Sample processing was performed by pipetting 200 µL of sample from each variant,  
113 placing it into liquid nitrogen for 2 min and adding 500 µL of water. Each sample was sonicated  
114 with an ultrasound needle for 2 min, shaken for 1 min, and centrifuged for 20 min at 25,000 g and  
115 at 4 °C. 200 µL of supernatant was taken from each sample and mixed with 200 µL of 10% TFA.  
116 The samples were again centrifuged for 20 min at 25,000 g and 4 °C. After centrifugation, the  
117 supernatant was analysed by HPLC-ED (Fig. 2).

#### 118 *Preparation of samples for selenium detection*

119 Samples of liver weighting 0.3 g and samples of blood weighting 0.5 g were disintegrated by dry  
120 method in a muffle furnace (LAC, Czech Republic) and mineralized in 2.5 mL concentrated nitric  
121 acid (Horky et al. 2016a). The scheme of preparation is shown in Fig. 2.

#### 122 *Determination of reduced and oxidized glutathione, and selenium*

Reduced and oxidized glutathiones were determined using high performance liquid chromatography with electrochemical detection (HPLC-ED). Experimental conditions were adopted from (Zitka et al. 2012). Selenium was determined on 280Z Agilent Technologies atomic absorption spectrometer (Agilent, USA) with electrothermal atomization under the conditions stated in (Horky et al. 2016a).

### *Statistics*

The data were processed statistically using STATISTICA.CZ, version 10.0 (Czech Republic), number of measurements were 3,  $P < 0.05$  were considered significant using ANOVA and Scheffe's method for the parameters GSH; GSSG; Se.

### **Results**

In the experiment, conventional (sodium selenite) and alternative forms of selenium (selenium nanoparticles), as the source of this element for the animal organism, were investigated. Oxidative glutathione, oxidized glutathione and selenium in blood and liver were selected as markers of oxidative stress. The level of oxidized and reduced glutathione was smaller increase in liver and blood, with the exception of GSH in liver samples. In the liver tissue, a significant decrease in the  $\text{Na}_2\text{SeO}_3$  group by 30% ( $P < 0.05$ ) was found together with both groups containing selenium nanoparticles, Se-49 by 34% ( $P < 0.05$ ) and Se-100 by 29% ( $P < 0.05$ ) (Fig. 3A). In the blood, a statistically significant reduction in GSH in all control groups was determined ( $\text{Na}_2\text{SeO}_3$  by 72%, Se-49 by 59%, Se-100 by 67%,  $P < 0.05$ ). Conversely, the increase in GSSG was found in  $\text{Na}_2\text{SeO}_3$  group by 17% ( $P < 0.05$ ), Se-49 by 51% ( $P < 0.05$ ) and Se-100 by 47% ( $P < 0.05$ ) (Fig. 3B).

Further, we determined content of selenium. In liver samples, a significant increase in selenium concentration in the  $\text{Na}_2\text{SeO}_3$  group was observed by 85% ( $P < 0.05$ ), Se-49 by 30% ( $P < 0.05$ ) and

146 Se-100 by 73% ( $P < 0.05$ ), in comparison to control group of rats (Fig. 4A). The level of selenium  
147 in the blood was also the highest in the  $\text{Na}_2\text{SeO}_3$  group. There was an increase by 240% ( $P < 0.05$ )  
148 against control. Other groups showed significant increase as well, Se-49 by 18% ( $P < 0.05$ ) and  
149 Se-100 by 64% ( $P < 0.05$ ) (Fig. 4B).

## 150 Discussion

151 In our experiment, the effect of an alternative source of selenium, selenium nanoparticles, was  
152 studied in terms of influencing the antioxidant potential of a rat organism. Antioxidant activity is  
153 an indicator of the ability of an entire body and selected organs to defend against free radicals.  
154 Reducing the antioxidant activity of the organism leads to an intensification of oxidative stress  
155 that affects the whole body, increases the risk of injury, reduces performance and deteriorates  
156 certain diseases.

157 At present, there is relatively little available reference on the use of nanoselenium in diet. In a  
158 study on reduction of radioactive gamma radiation, selenium particles were given at a dose of 20  
159 mg Se/kg of body weight per day (i.e. 3 mg Se/animal/day) and 0.1 mg Se/kg of body weight per  
160 day (0.015 mg Se/animal/day). The level of selenium and GSH was not affected (El-Batal et al.  
161 2012). In contrast, the selenium level was increased by 64% in the Se-100 group and GSH level  
162 decreased in all our experimental groups in blood. However, it should be noted the animals had  
163 not been exposed to gamma rays which certainly has an effect on the animal's antioxidant status.  
164 The effect of selenium nanoparticles applied to sugar carrier (glucose) was studied (Horky et al.  
165 2016a). Selenium particles were given at a dose of 0.02 mg Se/animal/day. After 10 days, an  
166 increase in GSH and total GPx activity in the blood was found which is inconsistent with our trial  
167 where GSH elevation did not occur. In another experiment (Hadrup et al. 2016) on rats, the effect  
168 of selenium and selenium nanocomponents addition (0.05 mg/kg bw and 0.5 mg/kg bw) was  
169 compared with the control group. The doses were put into feed as solutions using a gastric tube

every other day, and urine samples were collected. After 14 days, no toxic effects or no evidence of weight reduction compared to control were demonstrated.

In the past, rat experiments were conducted to compare the effect of organic and inorganic selenium. According to the authors (Sochor et al. 2012), addition of 1.5 mg of Se in organic form (yeast) increased GSH and GPx activity, when compared to sodium selenite. From this experiment, it appears that the addition of 1.5 mg may increase the antioxidant potential of animals without the occurrence of signs of toxicity. Other group of authors (Kominkova et al. 2015) stated the optimal amount of GSH and GSSG as 90% or 10%, respectively. In our experiment, higher levels of GSSG (oxidized form) were observed in all selenium addition groups. In the blood, the difference was the most significant. It is possible that our selected amount and form of selenium (1.2 mg/kg diet) has already had depression in the optimal GSH : GSSG ratio. However, our results correspond to the results in the study (Blahova et al. 2014), where measured concentrations in the liver ranged from 6 to 800 nmol/g for GSH and from 30 to 800 nmol/g for GSSG. Similar results for the liver were also recorded in study (Guan et al. 2003). For blood samples, we achieved a higher concentration GSH and GSSG than in (Guan et al. 2003; Horky et al. 2016a), the difference is most likely caused by another sample preparation and analysis itself.

## Conclusions

The experiment investigated the effect of selenium nanoparticles on the antioxidant status of laboratory rats. Alterations in reduced and oxidized glutathiones revealed marked changes in the antioxidant status based selenium treatment, however, we confirmed that nano-form of selenium has less negative effects than standard one. This leads us to support an idea to use nanoSe as an alternative source of selenium. It would be appropriate to test these selenium sources even at lower concentrations in order to avoid potential toxicity.

194 **Conflict of interest statement**

195 The authors report no conflicts of interest.

196

## 197 Captions for Figures

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

## 202 Figure 2

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

## 206 Figure 3

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

## 208 Figure 4

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

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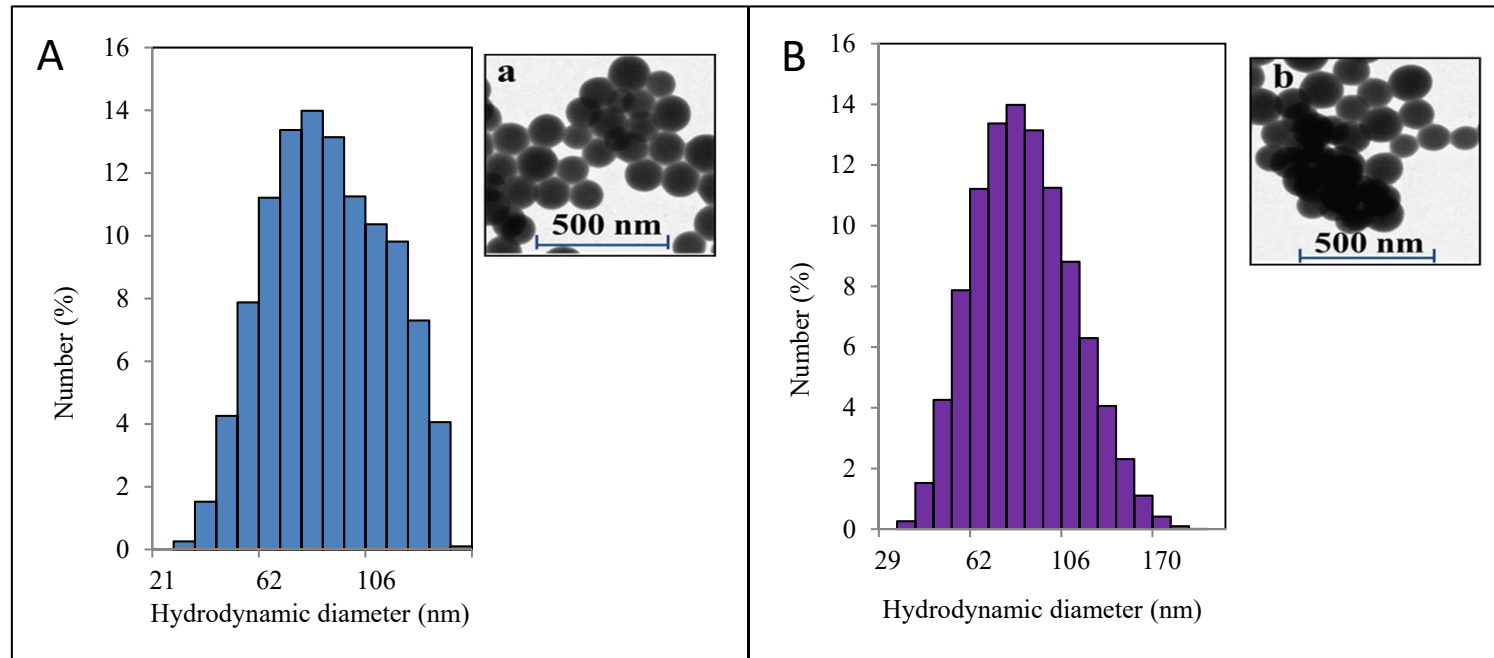
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# Figure 1(on next page)

## 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.

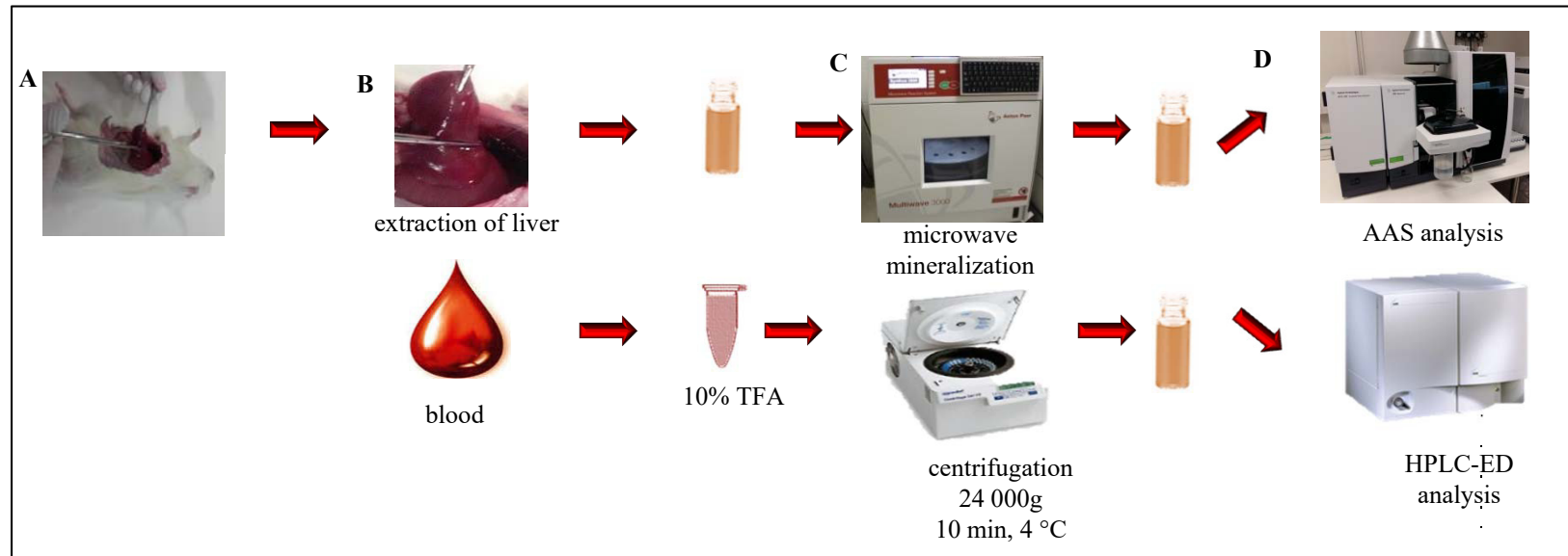


**Figure 1**

## Figure 2(on next page)

### 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.

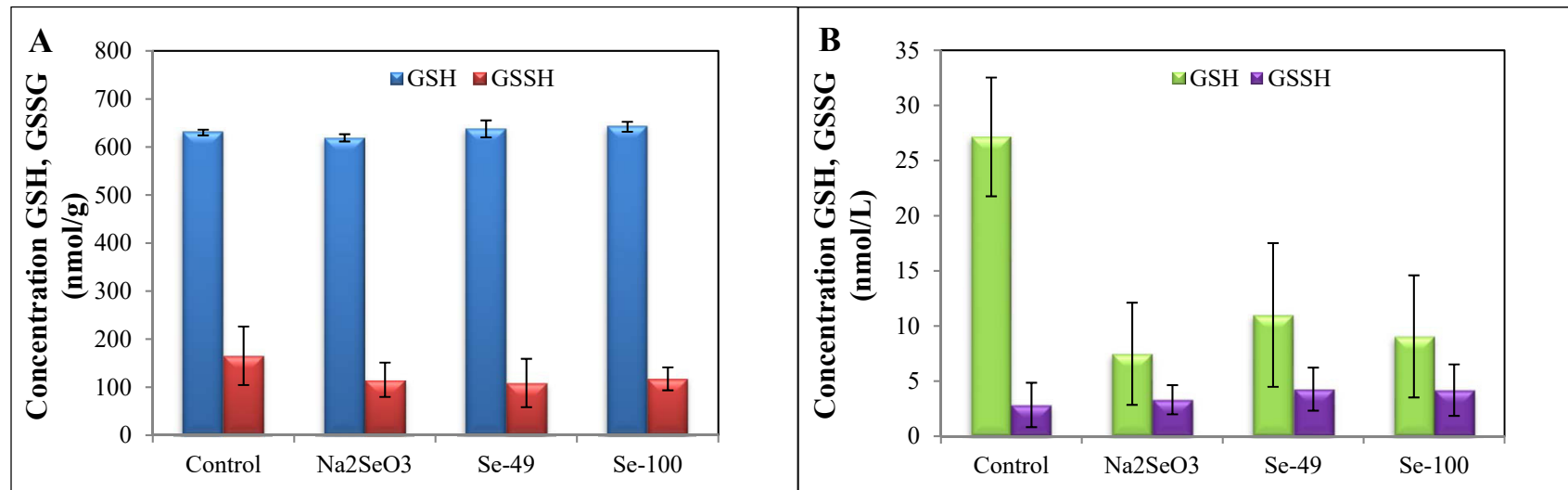


**Figure 2**

# Figure 3(on next page)

## Figure 3. Glutathiones

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

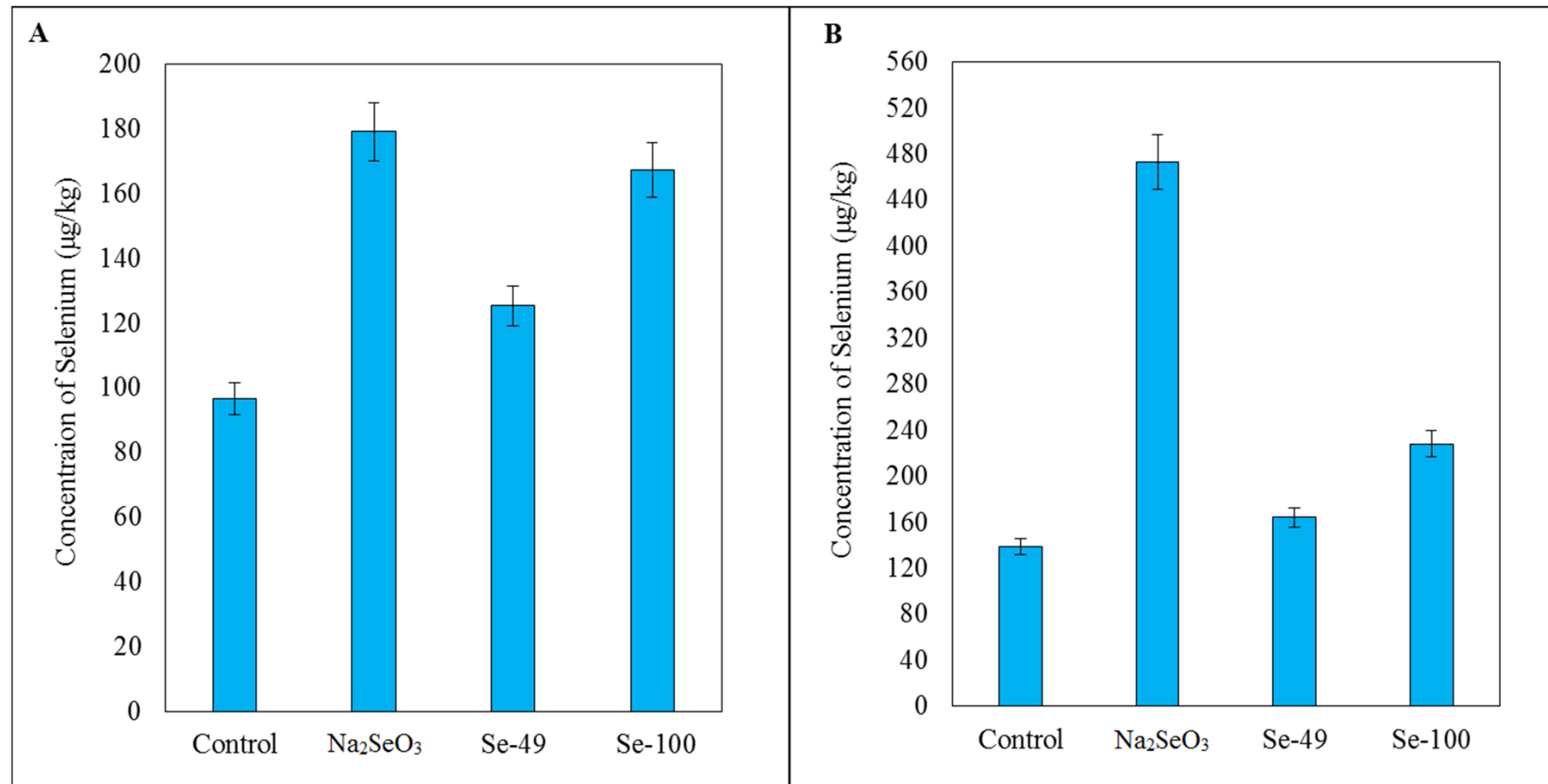


**Figure 3**

## Figure 4(on next page)

### Figure 4. Selenium

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



**Figure 4**