THE EFFECT OF ZINC ON THE CONCENTRATION OF REDUCED AND OXIDIZED GLUTATHIONE IN THE LABORATORY RATS ORGANISM

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Abstract: The aim of the experiment was to determine the effect of zinc nanoparticles (Zn NPs) on the antioxidant status of rats. The male of outbred Wistar albino rats were used in this experiment. The rats were divided into four groups. In each of group were stabled 5 males. The first group (Control) of rats served as control one without zinc administration. The second group (Zn -Phe) and the third group (Zn-Tyr) were administrated by zinc nanoparticles. In the fourth group of rats zinc oxide (ZnO) was dosed. After 30 days of the experiment, rats were sacrificed and the samples of blood and liver were analysed. Reduced (GSH) and oxidized (GSSG) form of glutathione was determined using high performance liquid chromatography with electrochemical detection (HPLC-ED) and total content of zinc was determined by atomic absorption spectrometry (AAS). In the analysis of liver, decrease of GSSG in all groups was observed. Statistically significant was decrease by 54% (P < 0.05) for ZnO group compared with control. The level of GSSG and total content of zinc in liver was without significant difference. In the whole blood, a significant decrease of GSH by 94% in the ZnO and by 65% in the Zn-Phe group was observed, compared with control group. The level of GSSG and total content of zinc in blood was without significant difference.

Key words: zinc, antioxidant status, rats, blood, liver

INTRODUCTION
Zinc (Zn) is an important essential trace mineral found in numerous enzymes, structural proteins, transcriptions factors and ribosomal proteins. It is involved in many physiological processes such as protein synthesis, carbohydrate metabolism and other biological reactions, which affects cellular functions (Zhao et al. 2016). Zinc deficiency is considered to cause an increased oxidative stress that leads to damage of biomolecules including DNA (Stenclova et al. 2016). The most often source of zinc is zinc oxide (ZnO). Due to its wide application, in cosmetics (UV-protection in sunscreens), in paints or as anticorrosive, antibacterial and antifungal agents, further increase of Zn nanoparticles use can be anticipated (Vankova et al. 2016).

Compared with ZnO, Zn NPs has a stronger chemical activity, oxidation reactions and the permeability on Zn NPs can help avoid adverse gastrointestinal reactions (Zhao et al. 2014). It may be used at lower doses in animal feed to provide better results than conventional Zn sources (Swain et al. 2016). Due to their small size, Zn NPs are readily absorbed and easily cross biological barriers, which make them promising candidates as diet additives. However, some studies have reported that Zn NPs cause toxicity, therefore, their safety and potency as diet additives for farm animals should be established (Zhao et al. 2016).

MATERIAL AND METHODS

Animals
The experiment was carried out in experimental facility on the Department of Animal Nutrition and Forage Production, Faculty of AgriSciences Mendel University in Brno. All tests were done in accordance with the act to protect animals against cruelty (No. 246/1992). Microclimatic conditions in...
laboratory limited by temperature were measured using DATALOGER S 3120 (Comet system, Czech Republic). The temperature was kept at 23 ± 1 °C. The same device was used for the monitoring of constant humidity. The air conditioning unit was set at a level of 60%. The photoperiod was driven according to scheme: 12 h per day and 12 h per night with maximal intensity 200 lx. The male of outbred Wistar albino rats strain were used in this experiment. The average weight of each animal was 235 ± 3 g.

The experimental animals were stabled on plastic cages with grates. The rats had free access to food and water ad libitum. The rats were sorted out to four groups. In each group were stabled 5 males. The first group (Control) of rats served as control one without zinc administration. The second group (Zn-Phe) and the third group (Zn-Tyr) were administrated by zinc nanoparticles (200 mg/kg of diet). In the fourth group of rats, ZnO (200 mg of zinc/kg of diet) was dosed. All groups were fed with monodi et (kibbled wheat) containing 32.2 mg of zinc per kg of body weight per day. The rats were sacrificed after 30 days of the experiment. The samples of whole blood and liver were obtained from animals and immediately subjected to the appropriate sampling analysis.

**Preparation of liver and blood samples**

**Liver:** A sample (2 g of liver, fresh weight) was deeply frozen by liquid nitrogen and 1.5 ml water. After that, sample was homogenized and vortexed for 10 min and centrifuged at 16 400 rpm (20 min, 4 °C). A volume of 100 µl of supernatant was taken and mixed with 100 µl of 10% trifluoroacetic acid (TFA). Subsequently the sample was centrifuged (20 min, 16 400 rpm, 4 °C). Supernatant was used for analysis.

**Blood:** A sample (200 µl of blood, fresh weight) was deeply frozen by liquid nitrogen and 500 ml water. Sample was vortexed for 1 min and centrifuged at 16 400 rpm (20 min, 4 °C). A volume of 200 µl was taken and mixed with 200 µl of 10% trifluoroacetic acid (TFA). After that, samples were centrifuged (20 min, 16 400 rpm, 4 °C). Supernatant was used for analysis. Samples were stored on the ice all the time.

**Determination of reduced and oxidized glutathione**

Reduced and oxidized glutathione was determined using high performance liquid chromatography with electrochemical detection (HPLC-ED). The chromatographic system consisted of two solvent delivery pumps operating in the range of 0.001–9.999 ml/min (Model 582 ESA Inc., Chelmsford, MA, USA), Zorbax eclipse AAA C18 (150 × 4.6; 3.5 µm particle size; Agilent Technologies, Santa Clara, CA, USA) and a CoulArray electrochemical detector (Model 5600A, ESA). The electrochemical detector includes three flow cells (Model 6210, ESA). Each cell consists of four working carbon porous electrodes, each one with auxiliary and dry Pd/H2 reference electrodes. Both the detector and the reaction coil/column were thermostated. The sample (20 µl) was injected using autosampler (Model 542 HPLC, ESA). Samples were kept in the carousel at 8 °C during the analysis. The column was thermostated at 35 °C. Mobile phase consisted of 80 mM TFA (A) and methanol (B). The compounds of interest were separated by the following linear gradient: 0 → 14.5 min (4% B), 14.5 → 16 min (5% B), 16 → 22 min (98% B), 22 → 31 min (4% B). Mobile phase flow rate was of 1 ml/min, working electrode potential 900 mV. Time of analysis was 31 min (Kominková et al. 2015).

**Determination of zinc**

A 10 µl of blood were pipetted into digestion vials and 10 mg of homogenized liver were weighed into digestion vials. Nitric acid suprapure and hydrogen peroxide (30%) were used as digestion mixture. A 500 µl of volume of digestion mixture was used (300 µl HNO3 and 200 µl H2O2). The samples were digested by Microwave 3000 (Anton Paar GmbH, Austria) rotor MG-65. Microwave power was 100 W in the main part of the programs (for 30 min, 140 °C). Zinc was determined by ContrAA 700 (Analytik Jena, Germany) atomic absorption spectrometer for flame and hydride technique. The spectrometer was operated at 213.83 nm resonance line (Horky et al. 2016, Horky et al. 2013).

**Statistic**

The data were processed statistically using STATISTICA.CZ, version 10.0 (Czech Republic). Significance was determined by examining the basic differences among groups using ANOVA and
Scheffé’s test for the parameters GSH; GSSG; Zn. Differences with P < 0.05 were considered significant.

RESULTS AND DISCUSSION

In the experiment, the influence of different forms of zinc (ZnO, Zn-Phe NPs, Zn-Tyr NPs) on the antioxidant status of rats was observed. Samples of whole blood and liver were analysed. The first observed parameter was the total level of zinc. No statistical significant differences were detected. In the liver, concentration of zinc was similar in all groups (Figure 1A). In the whole blood, there was increase by 21% in ZnO group and both groups with Zn NPs were decreased compared with control group (Figure 1B). The results of liver samples are in agreement with Horky et al. (2016) and Stenclová et al. (2016), but in their experiments, the content of zinc in blood was decreased in groups with Zn NPs. This could be caused by the use of different zinc modifications and shorter time of experiment.

Figure 1 The influence of Zn nanocomplexes in (A) liver, (B) blood on zinc concentration.

Another parameter, which indicates the antioxidant potential of the organism, was level of GSH and GSSG. In the liver a significant increase in the GSH level by 3% (P < 0.05) was found in the Zn-Phe group compared with control. In the case of GSSG, decrease by 54% in ZnO group with significant evidence was observed. On the other hand a decrease was observed for all experimental groups (Figure 2A).

Figure 2 The influence of Zn nanocomplexes in (A) liver, (B) blood on the level of reduced (GSH) and oxidized (GSSG) form.

In the analyses of whole blood, a significant decrease of GSH was measured in the ZnO group (by 94%, P < 0.05) and Zn-Phe group (by 65%, P < 0.05) compared with control. Lower level of GSH was also observed in the Zn-Tyr group (by 41%) but without statistical significance. The whole blood
samples showed increase in the GSSG concentration but not statistically significant (Figure 2B). Kominková et al. (2015) indicates the optimal GSH : GSSG ratio is 90 : 10%. In our experiment, in liver, increase of GSH and decrease of GSSG in both groups with zinc nanoparticles was measured.

CONCLUSION
The experiment was focused on the use of zinc nanoparticles and zinc oxide in the diet of rats. The antioxidant status was observed by GSH and GSSG determination. Both forms of zinc had the effect on the level of reduced and oxidized glutathione in a whole blood and the liver. The total level of zinc was decreased in liver and also blood samples, but without significant difference.

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REFERENCES


