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ACTIVITY OF SUPEROXIDE DISMUTASE IN RAT OVARIAN FRAGMENTS EXPOSED TO MOLYBDENUM AND SILVER IN VITRO

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ABSTRACT

The aim of this study was to determine the activity of superoxide dismutase (SOD) in rat ovarian fragments cultured *in vitro* after molybdenum (Mo) and silver (Ag) administrations. Ovarian fragments were incubated with Mo (ammonium molybdate, $(NH_4)_6.Mo_7O_{24}.4H_2O$) and Ag (silver nitrate, $AgNO_3$) as follows: 0.13 mg.ml^{-1} in E1 group, 0.17 mg.ml^{-1} in E2, 0.33 mg.ml^{-1} in E3, 0.5 mg.ml^{-1} in E4, and 1.0 mg.ml^{-1} in E5 for 24 hours. The group without any addition served as the control. Activity of SOD in ovarian fragments significantly decreased in all experimental groups when compared to the control suggesting uncontrolled overproduction of reactive oxygen species (ROS) and failure in antioxidant defence system. Trace elements can adversely affect animal reproductive system and its functions, through either direct or indirect effects on oxidative stress induction.

Keywords: Rats, ovarian fragments, molybdenum, silver, SOD

INTRODUCTION

Reactive oxygen species (ROS) are generated as a part of normal oxidative metabolism, however, cell death or cellular damage can occur from their excess production **(Droge, 2003)**. During oxidative stress, the production of ROS overwhelms the ability of anti-oxidant defence pathways to maintain redox equilibrium within the cell **(Wells *et al.*, 2009).** Superoxide dismutase (SOD) catalyses the dismutation of superoxide into hydrogen peroxide (H_2O_2) and oxygen, thus maintaining low steady-state levels of superoxide. Because excess superoxide is toxic, SOD is ubiquitously present in different organelles within the cells **(Fridovich, 1997)**. When SOD activity is reduced, an accumulation of superoxide radicals can result **(Halliwell, 2011).**

Molybdenum (Mo) is essential trace element for plants, animals and humans. It improves glucose homeostasis by inducing the insulin receptor tyrosine kinase activity in hepatocytes **(Reul *et al.*, 1997; Bersényi *et al.*, 2008).** Mo deficiency was found to decrease the conception rate, fetal survival and the number and viability of offspring of animals **(Rajagopalan, 1988).** Toxicosis (molybdenosis) caused by Mo exposure is essentially a secondary copper deficiency **(Suttle, 1991).** High dietary Mo content can generate free radical processes or reactive intermediates **(Bersényi *et al.*, 2008).**

Silver (Ag) is white transitional element found in the environment. Low concentration of Ag is present in the animal body (**Lansdown, 2006).** This metal has been used for centuries as an antimicrobial agent to reduce bioburden and prevent infection **(Edward-Jones, 2009).** Increasing use of silver in recent years has led to concern as to the safety aspects of the metal and potential risks associated with absorption of the biologically active Ag into the human body **(Lansdown, 2006).** Our previous study revealed that exposure of porcine blood cells to silver *in vitro* caused changes and imbalance in blood elements. Significant decrease in erythrocytes, haemoglobin content and haematocrit was observed **(Capcarová *et al.*, 2011)**.

The aim of this study was to analyse the effect of molybdenum and silver on the activity of SOD in rat ovarian fragments *in vitro*.

MATERIAL AND METHODS

Preparation, culture and processing of rat ovarian fragments

Ovaries were obtained from adult rats 4 months of age slaughtered by decapitation at follicular stage of the ovarian cycle (determined by visual inspection of the ovaries) without visible reproductive abnormalities. Decapitation was performed under ether anaesthesia according to EU and Slovak guidelines of performance animal experiments. Isolated ovaries were transported to the laboratory in containers at 4ºC and washed in sterile physiological solution. Thereafter ovaries were cut by razor blade into fragments approx. 2 mm size. Ovarian fragments (n = 48) were washed in sterile DMEM/F12 1:1 medium (BioWhittaker™, Verviers, Belgium) and incubated for 24h in culture plates (Nunc™, Roskilde, Denmark, 1 ml/well) in the same medium with 10 % fetal calf serum (BioWhittaker™, Verviers, Belgium),1 % antibiotic–antimycotic solution (Sigma, St. Louis, Mo, USA), with Mo (ammonium molybdate, $(NH_4)_6.Mo_7O_{24}.4H_2O$, Slavus Bratislava, Slovak Republic) and Ag (silver nitrate, $AgNO_3$; Slavus Bratislava, Slovak Republic) as follows: 0.09 mg.ml^{-1} in E1 group, 0.17 mg.ml^{-1} in E2, 0.33 mg.ml^{-1} in E3, 0.5 mg.ml^{-1} in E4, and 1.0 mg.ml^{-1} in E5. The group without any addition served as the control. After 24h of culture the media from wells were aspirated and cells from plated were manually smashed and lysate was obtained.

SOD analysis

The activity of antioxidant enzyme SOD of rat ovarian fragments was assayed by spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc, USA) using antioxidant RANDOW kits (Randox Labs, Crumlin, UK) according to the manufacturer's instruction.

Statistical analysis

Each experimental group was represented by six culture wells of ovarian fragments (n=48). Significance of differences between the groups was evaluated by one-way ANOVA using statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as means ± SD. Differences were compared for statistical significance at the P - level less than 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

Female reproductive functions can be affected negatively by exposure to toxic chemicals **(Mlynarcikova *et al*., 2005; Kolesarova et al., 2010).** Our previous studies showed some changes in haematological and antioxidant parameters in animal cells after an exposure by various environmental contaminants **(Capcarová *et al*., 2009; Petruška *et al*., 2012; Capcarova *et al*., 2013a; Zbyňovská *et al*., 2013).** When ROS are overproduced, oxidative stress may develop in the body **(Jones, 2008)**. SOD serves as front-line antioxidant defence **(Scandalios, 2005).**

In the present study decrease in SOD activity was observed in all experimental groups when compared to the control. The significant differences (P<0.05) were found between the control and E2, E3, E4, and E5 group (Fig 1). The decrease corresponded with the dose of molybdenum. **Bersényi *et al.* (2008)** found higher production of ROS, consequently alteration in malondialdehyde and glutathione peroxidase activity after dietary molybdenum in rabbits. **Arthington *et al.* (1996)** observed decrease in SOD activity in heifer in Mo-supplemented group. Similarly to our previous study, molybdenum treatments significantly decrease the activity of SOD in hens' granulosa cells **(Capcarova *et al*., 2012)** and caused changes and imbalance in immune cells (**Capcarova *et al*., 2013b)**.

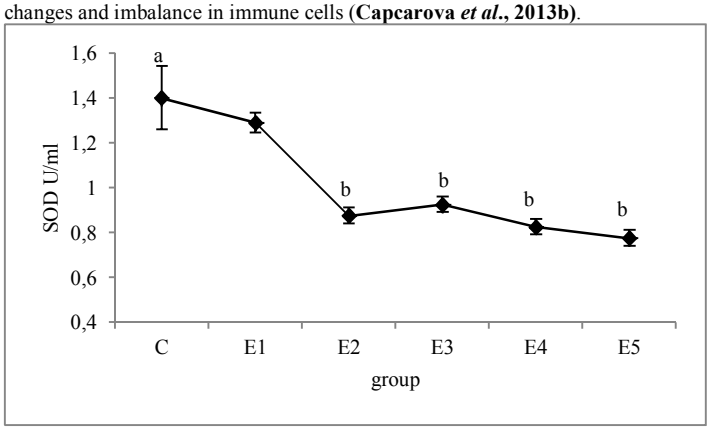

Figure 1 The effect of Mo treatment on SOD activity in rat ovarian fragments
C- control group, E1-E5 – experimental groups with various doses of Mo, SOD – superoxide dismutase, a-b- means significant differences (P<0.05), one-way ANOVA

In this study the activity of SOD decreased also after Ag treatment, however the decrease was not as significant as in case of Mo. The significant difference (P<0.05) was found between the control and the group with the highest dose of Ag (Fig. 2). **Avalos *et al.* (2013)** found that Ag nanoparticle caused disturbance in cellular antioxidant status and slight inactivation of SOD activity, and significantly increased the reactive oxygen radicals **(Kim et al., 2011)**.

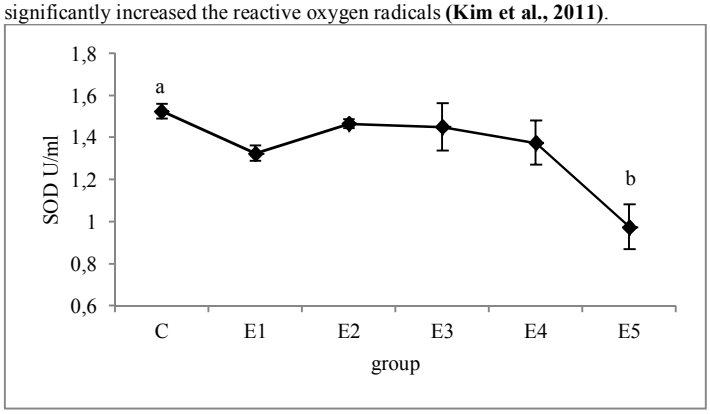

Figure 1 The effect of Ag treatment on SOD activity in rat ovarian fragments
C- control group, E1-E5 – experimental groups with various doses of Ag, SOD – superoxide dismutase, a-b- means significant differences (P<0.05), one-way ANOVA

CONCLUSION

The results could indicate the presence of oxidant/antioxidant imbalance in rat ovarian fragments due to various doses of Mo and Ag. Our results demonstrated that Mo and Ag dose were probably too high as SOD activity decreased in all experimental groups.

Results of this study provide a foundation for further analysis and researches of heavy metals impact on living cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on antioxidant status of cells.

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