## 7. SUMMARY

Acrylamide (AA) is a dangerous chemical substance which can be found in food. AA biotransformation takes place mainly in the liver. AA and its metabolites react with hemoglobin (Hb), and that is why Hb can have an impact on blood morphology and functions. Furthermore, AA biotransformation is connected with kidney and spleen functions. These organs are important in metabolism and excretion of AA and they have a great impact on blood function. AA metabolism is accompanied by the production of reactive oxygen species, activation of antioxidative system, which influences the redox balance, and the functions and structure of organs important for xenobiotic metabolism. AA and its metabolites can result in the oxidation of biological elements, and can damage the cell and genetic material of organs engaged in AA metabolism.

In the following thesis, it was assumed that the intake of metal cations, which play an important role in the antioxidative functions, can have an impact on the reduction of AA toxic action. Magnesium ($Mg^{2+}$) and zinc ($Zn^{2+}$) ions are cations which are cofactors of several enzymatic substances and which take part in xenobiotic biotransformation and neutralization of reactive species. The supplementation of $Mg^{2+}$ and $Zn^{2+}$ can change the AA impact on blood morphology and the functions and structure of liver, kidneys and spleen.

The aim of the thesis was the assessment of the AA, $Mg^{2+}$ and $Zn^{2+}$ influence on blood morphological parameters, that is: concentration of white blood cells (WBC), lymphocytes (Lymph), red blood cells (RBC), Hb and hematocrit (HTC) and survival of bone marrow cells. Furthermore, the aim of this work was to assess the antioxidative status by marking glutathione reduced (GSH) concentration and activity of antioxidative enzymes, that is superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in liver, kidney and spleen of mice after AA, $Mg^{2+}$ and $Zn^{2+}$ intake; marking malondialdehyde (MDA) – lipid peroxidative product – in liver and kidneys; assessing the caspaze-3 expression and structural changes in liver, kidneys and spleen of mice after AA, $Mg^{2+}$ and $Zn^{2+}$. Moreover, the study contains the assessment of the liver enzymes activity: alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) in liver of mice after the AA, $Mg^{2+}$ and $Zn^{2+}$ intake.

Within the research two *in vivo* experiments with mice, line Swiss, were carried out. In the first experiment the mice were given AA in dose of 20 mg/kg bw and 40 mg/kg bw, and $Mg^{2+}$ ions in dose of 5 mg/kg bw. In the second experiment, the mice were given AA (the same doses as in the first experiment), and $Zn^{2+}$ ions in dose of 3.5 mg/kg bw. AA, $Mg^{2+}$ and

$Zn^{2+}$ were given *per os*, in water solution, for 10 days. The mice from the control group were given drinking water. The blood for morphology was taken form tail vein, after 24h from the first intake and after 24h from the last cations and AA intake. After the euthanasia, liver, kidney and spleen were taken out for biochemical and histological examination. Breeding and the analysis of bone marrow cell viability were carried out in the in vitro experiment on bone marrow cells taken from rabbit's femur. The AA impact (AA concentration 1 mM, 2.5 mM, 5 mM and 10 mM), and the impact of $Mg^{2+}$ (40.8 mg) and $Zn^{2+}$ (0.65 mg) ions on the bone marrow were assessed by MTT test.

Haematological analysis was made in blood with the use of haematological analyser. The measurement of MDA, GSH, GSH-Px, SOD, CAT and enzymatic indicators of liver functions – ALP, ALT and GGT activity – were made on the basis of spectrophotometric technics of absorbance measurement. The indications of caspase-3, basic and DAPI staining of histological preparation in liver, kidney and spleen of mice were made with the use of basic and immunohistochemical staining (IHC).

The results of the research have shown that AA influences blood parameters to a small extent, that is: WBC, Lymph, RBC, Hb and HTC. $Mg^{2+}$ and $Zn^{2+}$ supplementation caused that the analysed morphological blood parameters of mice after AA intake returned to control parameters. It was observed that AA intake in *in vitro* bone marrow cells cultures reduced their viability. The intake of $Mg^{2+}$ ions partly eliminates this effect. AA caused significant increase of MDA concentration and caspase-3 activity, changes in the activity of ALP, ALT and GGT and histopatological changes in liver, kidney and spleen. These changes were accompanied by redox imbalance. The GSH concentration decrease and changes in GSH-Px, SOD and CAT activity in liver, kidney and spleen, may indicate that organ damage mechanism caused by AA is connected with oxidative stress. $Mg^{2+}$ and $Zn^{2+}$ supplementation caused partial return of GHS concentration and activity of liver enzymes to control parameters. The observed changes show protective role of $Mg^{2+}$ and $Zn^{2+}$ ions during AA toxic effect.

The research shows that AA had moderate effect on blood parameters. The changes in blood morphology observed after AA, $Mg^{2+}$ and $Zn^{2+}$ intake can be related to viability of bone marrow cells. $Mg^{2+}$ and $Zn^{2+}$ supplementation seems to be beneficial to the general health condition, because after the supplementation, the analysed blood parameters of mice after AA partly returned to control parameters. The observed changes were accompanied by redox imbalance which can prove that organ damage mechanism after AA is connected with oxidative stress. $Mg^{2+}$ and $Zn^{2+}$ supplementation caused decrease in the toxic effect of AA in

all the examined organs which also confirms the oxidative character of damage observed in the organs of mice after AA.