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Glutathione protects liver and kidney tissue from cadmium- and lead-provoked lipid peroxidation

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Abstract: Cd and Pb represent a serious ecological problem due to their soluble nature, their mobility and ability to accumulate in the soil. The exposure to these heavy metals can originate from different sources (drinking water, food and air), and they can enter into the human body through the respiratory and digestive systems. The effects of glutathione on Cd and Pb accumulation and lipid peroxidation effects in the liver and kidneys of heavy metal intoxicated rats were investigated. The content of the marker of lipid peroxidation, malondialdehyde, was increased several fold in the tissues of the exposed animals, the effects being more pronounced in the liver. The treatment of intoxicated animals with glutathione drastically suppressed lipid peroxidation. The obtained results imply that the application of glutathione may have a protective role in heavy metal intoxication by inhibiting lipid peroxidation. However, precaution should be exercised when Cd is considered, since it seems that glutathione promotes Cd accumulation in the liver.

Keywords: cadmium; lead; glutathione; lipid peroxidation; malondialdehyde.

INTRODUCTION

Heavy metals are toxic, non-biodegradable and have a very long half-life in the soil.[1] They enter living systems *via* food chains, water or air.[2] For this reason, metals, as polluters in the working and living environment, represent a very serious health and ecological hazard.

Volcanic activity is one of the reasons for the periodical increase in cadmium concentrations in the living environment, primarily in the air. The constant sources of cadmium contamination are related to its application in industry as a

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corrosion reagent, a stabilizer in PVC products and car tires, colour pigments and nickel–cadmium batteries. Groups which are highly exposed to its effect include not only factory workers, as even the population living within a 2 km radius of a cadmium source are considered to be at a risk of high exposure, whereas people living within a 2 to 10 km radius are considered to be at a risk of medium high exposure.[3] Smoking is a significant source of intoxication, as cadmium is inhaled during the process. Through cigarette smoke, 50 % of cadmium is absorbed into the lungs and transferred into the circulatory system during active smoking.[4] Absorption mainly takes place by means of the respiratory tract, and to a smaller extent *via* the gastro–intestinal tract, while an insignificant amount is absorbed transcutaneously. When it enters the body, cadmium is transported into the bloodstream *via* red blood cells and albumin.[5] Although cadmium is spread through the bloodstream throughout the entire body, it is mostly accumulated in the kidneys and liver. Cadmium excretion from the body is slow and is performed *via* the kidneys and bile, saliva and milk during lactation.[6]

The sources of lead contamination include metallurgic gases which are a part of the non-ferrous extractive metallurgy, the metallurgy of iron and steel and also exhaust gases from the chemical industry and traffic, industrial waste, mine and landfill waters.[7] The most exposed are the workers in lead smelters and foundries, recycling plants, paint, ceramic, glass, battery and ammunition industry. From the atmosphere, soil and water (both surface and underground) lead is introduced and retained in plants, thereby finding its way into human body *via* the food chain and drinking water. Lead can be introduced into the body through the skin and digestive tract.[8] Once it enters the body, lead circulates through it mainly connected to erythrocytes, and to a lesser extent to plasma albumin, and least of all in ionic form or tied to low molecular proteins. It is mostly accumulated in the bones, then the liver, kidneys, spleen, nervous tissue and muscles. From the human body it is usually extracted through urine, slightly less through the mucous membrane of the digestive tract, gall, hair, nails, sweat and milk.

Although cadmium is not a redox active metal and cannot actively participate in Fenton's reaction or the creation of reactive oxygen species (ROS), it indirectly leads to oxidative stress and consequent damage to body structure, due to its ability to bind thiol (–SH) groups which leads to the depletion of the central antioxidant – glutathione (GSH).[9] Recent research has shown that cadmium causes oxidative damage to DNA, proteins and lipids. At the molecular level, the action of lead can be seen in the increased production of reactive oxygen species.[10] The lead(II) ion can accelerate oxidation of oxyhaemoglobin to methaemoglobin (with increased formation of ROS). In addition, lead combines with thiol groups on proteins and, at high concentrations, causes GSH depletion. Lead may directly be attached to a cell membrane, thus increasing the sensitivity of the membrane to the process of lipid peroxidation.

Lipid peroxidation is oxidative damage that affects cell membranes, lipoproteins and other molecules containing lipids under conditions of oxidation stress.[11–13] Lipid peroxidation is a self-propagating chain reaction caused by the reaction of free radicals on unsaturated fatty acids in cell membranes. Lipid peroxidation reduces the fluidity of biological membranes resulting in increased permeability for uni- and divalent ions and deactivation of membrane enzymes.[14,15] The fragmentation of fatty acid chains to the level of intermediates of the aldehyde type and short chain volatile hydrocarbons leads to the loss of membrane integrity, while the rupture of lysosome membranes leads to the release of hydrolysis enzymes which further damage cells.[16] The process of lipid peroxidation initiates the death of cells.[17,18]

Malondialdehyde (MDA) is the final product in the lipid peroxidation process, and may be presented in enol form:

$$CH_2(CHO)_2 \rightarrow HOCH{=}CH{-}CHO$$

This compound is a biomarker of oxidative stress in the body or individual organs. MDA has very strong cytotoxic effects. The reaction between proteins, RNA, DNA, or phospholipids may cause the modification of these substrates and damage to cell membranes and intracellular molecules.[19] MDA when attached to DNA creates so-called “DNA radicals”, which are responsible for mutation. MDA is a reactive potential mutagen and carcinogen. It also inhibits numerous thiol-dependent enzymes, such as glucose-6-phosphatase, Na^+, K^+-ATP-ase, adenylate cyclase and Ca^{2+}ATP-ase.[13] The degree of lipid peroxidation is evaluated by measuring the level of MDA in different tissues.[20]

GSH is a tripeptide L-γ-glutamyl-cysteinyl-glycine, which makes up 90 % of the overall non-protein sulphate compounds of the cell and is an essential cofactor of some enzymes (glutathioneperoxidase, glutathione S-transferase, glutathione transhydrogenase, glutathione reductase). GSH is a biological redox agent in erythrocyte metabolism and plays a role in the transfer of amino acids.

GSH is widespread in human and animal tissues, and plants and microorganisms. Intra-cell concentrations of 0.1–10 mM make GSH one of the most frequent thiol compounds.[21] GSH as a thiol compound is an antioxidant found within a cell.[22] Based on the results obtained upon defining the content of GSH in mosquitoes, flies, mice, rats and humans, a hypothesis was formed that its concentration reduces with age, which is the possible key to ageing and the appearance of different pathological states.[23]

Herein, the accumulation of Cd and Pb and the related lipid peroxidation in liver and kidney tissue of model animals (albino Wistar rats) is investigated. The study is focused on the effects of GSH on these processes. The protective role of glutathione was examined by measuring the MDA concentrations.

EXPERIMENTAL

The model system. The study was realized on female white (albino) Wistar rats, 2 months old with an approximate weight of 200±20 g. The animals were kept in groups, in metal cages under laboratory conditions with ventilation systems and an environmental temperature of t = 22±2 °C.[24] The experimental animals were bred under laboratory conditions with procurable water and food *ad libitum*, in vivarium at the Faculty of Medicine in Niš, divided into 6 groups of 6 animals. The groups were designated as Group I, II, III, IV, V and VI.

Heavy metal intoxication and the addition of a supplement. The first group of experimental animals was kept on a normal diet and led a normal life (the control group). The experimental animals from Group II and III were intoxicated by subcutaneous injection with an overall dose of 0.18 mg of cadmium in the form of cadmium(II) chloride in saline solution (0.9 % NaCl).[25] One day following intoxication by cadmium, the animals from Group III received a dose of glutathione in a molar ratio of metal:GSH = 1:2.[26] The animals from Groups IV and V were intoxicated with an overall dose of 21 mg of lead in the form of lead(II) acetate in saline solution in 7 equal doses over a period of 21 days.[27,28] One day following intoxication *via* lead, the animals in Group V received a dose of glutathione in a mol ratio of metal:GSH = 1:2.[26] The animals in Group VI were treated only with glutathione for the duration of the experiment. All the used reagents were of p.a. purity, and manufactured by Sigma–Aldrich (Steinheim, Germany). The experimental procedure was performed in accordance with the ethical scientific work code.

Preparation of the biological material for analysis. Ketalar anaesthesia (35 mg kg^{-1} of body weight) was performed on the rats after a certain number of days. Following laparotomy, the liver and kidneys were removed and, after washing in saline solution, were encapsulated, frozen at –20 °C and later used for making homogenates. Homogenization was performed on ice using a Teflon pestle Ultra Turrax® IKA® Tl8 basic homogenizer (IKA). From 10 % of the liver and kidney tissue homogenates, prepared in ionized water, samples were extracted for the following analyses: the determination of malondialdehyde concentrations and the definition of Cd and Pb concentrations in the organs.

Determination of the concentration of the lipid peroxidation product (TBARS) in the tissue homogenates. The level of lipid peroxidation in the tissues is expressed as the concentration of thiobarbituric acid reactive substances (TBARS) in the tissue homogenates and was defined by means of a spectrophotometric method.[29]

Determination of the metal concentration in the tissue homogenates of the kidneys and liver. The content of toxic metals in the tissue homogenates was determined by potentiometric stripping analysis (PSA).[30]

Statistical analysis. All of the obtained results are presented as average values of all the samples in one group, average ± standard deviation (*SD*). For statistical processing of the results, the Student t-test for independent samples was used. The statistical importance of the tests was defined as p<0.01.

RESULTS AND DISCUSSION

The results of the content of lead and cadmium in the analyzed kidney and liver tissue homogenates, with and without the GSH supplementation are shown in Fig. 1. It can be observed that both Cd and Pb accumulate to a higher level in the liver in comparison to kidneys. This can be attributed to a higher lipid content and detoxifying function of liver. GSH showed biased effects on the metal accu-

mulation. It promoted the accumulation of Cd in liver, but suppressed Pb accumulation in both the studied organs. This may be related to the binding of Cd and Pb to GSH, which may alter their metabolism in the blood.

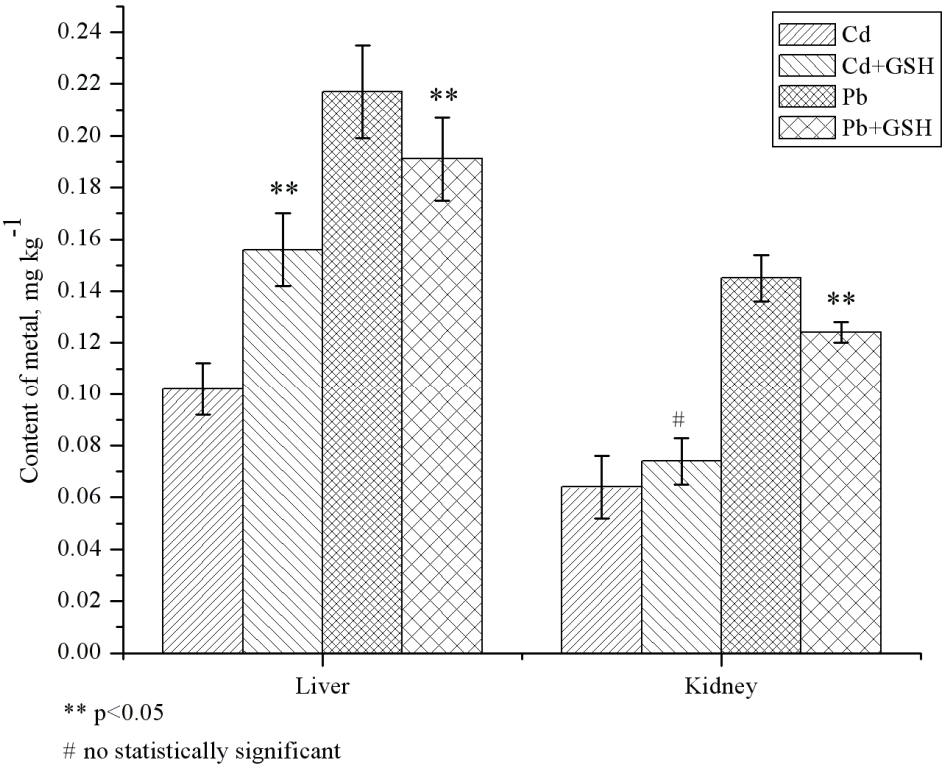

Fig. 1. The content of Cd and Pb metals in the homogenates of the analyzed liver and kidney samples, in the presence and absence of GSH.

The results obtained from monitoring the toxic effects of cadmium in the analyzed tissue samples obtained through the content of MDA are shown in Fig. 2. The results in Fig. 2 show that under conditions of cadmium intoxication, the level of MDA was increased when compared to the level found in the liver and kidneys of the control group of animals. The results show that after cadmium intoxication, the level of TBARS was significantly increased. A direct consequence of exposure to cadmium *in vivo* is probably the depletion of physiological antioxidants, such as reduced glutathione and proteins which contain –SH groups, as one of the mechanisms of cadmium toxicity. In the experimental group of animals, where along with cadmium, GSH was applied as a supplement, the concentration of MDA was significantly reduced in comparison to the group of animals intoxicated with cadmium. The application of GSH along with cadmium

leads to a reduction in the TBARS level, probably because of the intake of supplements with –SH groups. Cadmium attaches to the active glutathione centre (–SH) and donor atoms (–S, –N and –O), thus reducing its pro-oxidant effect. Based on these research results, it may be concluded that the level of lipid peroxides is significantly reduced in the case when GSH supplement was added one day following intoxication of the rats with cadmium.

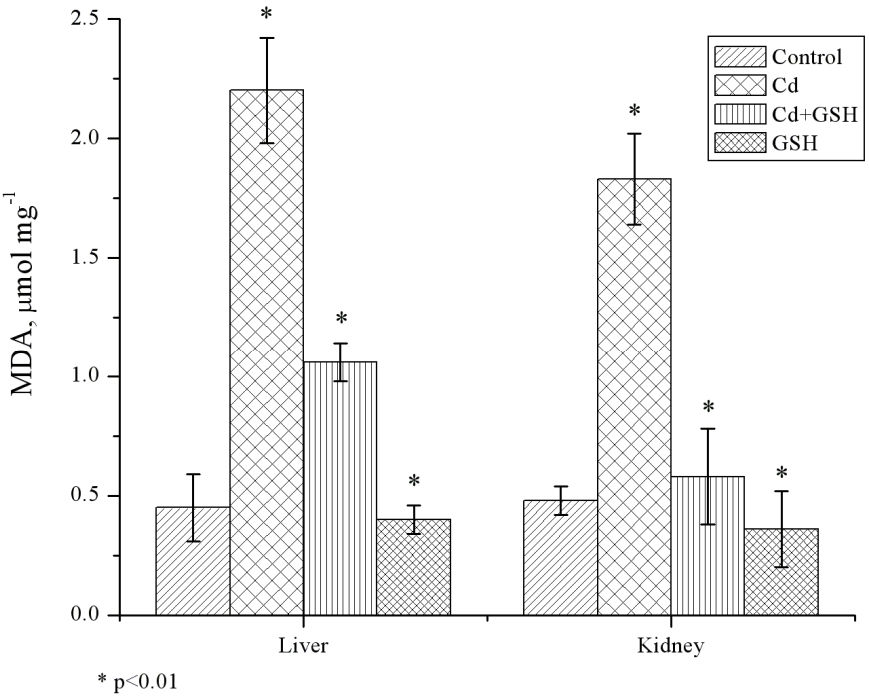

Fig. 2. The content of MDA in the homogenates of the analyzed tissue samples in the case of acute cadmium intoxication, in the presence and absence of GSH.

As a consequence of chronic Pb intoxication, humans suffer from liver disorders that cause the deactivation of important enzymes with active sulphydryl groups. An entire group of disorders occur in the hepatocytes, including the synthesis of haem. The synthesis disorder of haem creates free radicals which further destroy liver cells. Intoxication with Pb reduces kidney circulation which is caused by changes in the small blood vessels, leading to lead nephropathy.[31] Persons who are exposed to Pb in their line of work suffer from kidney disorders, which lead to increased levels of urea, creatinine and urine acids in their serum, higher than the reference values.

The results of the determination of MDA concentrations in Wistar rats after intoxication by lead are presented in Fig. 3.

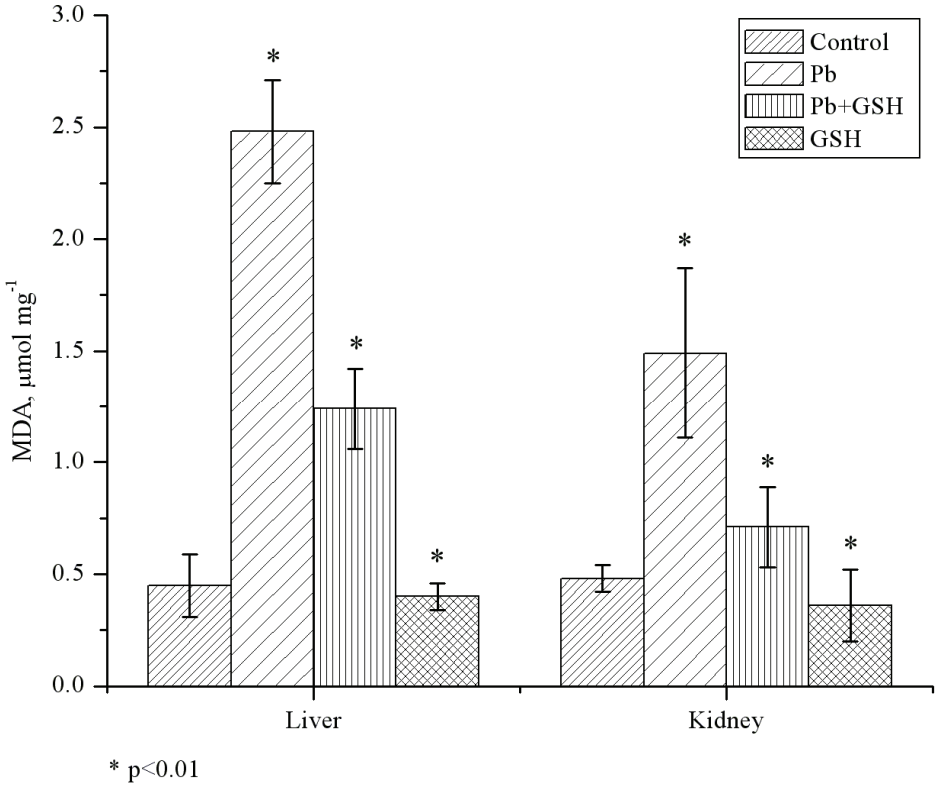

Fig. 3. The content of MDA in the homogenates of the analyzed tissue samples in the case of acute lead intoxication, in the presence and absence of GSH.

Under lead intoxication, the level of MDA in the liver and kidneys increased as compared to the level in the control group. Lead probably directly or indirectly increases the number of reactive oxygen species and reduces the antioxidative system of cell defence, which causes an imbalance between free radicals and antioxidants. It is known that lead has a toxic influence on cell membranes and its functions (for example mitochondrial membranes, endoplasmic reticulum membranes, lysosome membranes and plasma membrane). The cell membrane, in addition to other chemical compounds, contains fatty acids. The presence of double bonds in them weakens the allylic C–H bond in the =C–H system, which leads to an easier release of H. As a result, fatty acids with <2 double bonds are more resistant to oxidative stress, unlike unsaturated ones with >2 double bonds in their molecule. Upon the exposure of essential fatty acids to lead, the concentration of the final product of oxidative stress, MDA, increases with increasing number of double bonds, Lead may directly be bound to a cell membrane, which results in sensitivity of the membrane to lipid peroxidation.[32]

In the group of experimental animals to which, in addition to lead, glutathione was applied as a supplement, the MDA concentrations were significantly reduced in comparison to the group poisoned by lead, as can be seen in Fig. 3. The reduction of the toxic effect of lead in the presence of GSH is the result of the interaction between Pb^{2+} and donor atoms of GSH, which shows that the time difference between the input of this metal and GSH, one day after intoxication with metal, indicates the possibility that GSH and the food that contains it may be a good supplement for the purpose of reduction of the toxic effects of heavy metal, especially those of lead.

The presence of a GSH supplement significantly reduces the toxic effects caused by Cd and Pb intoxication.

Increased values of MDA, as a biochemical marker of oxidative disorder of cell membranes, indicate an increased process of lipid peroxidation, which was found in the kidney tissue homogenates due to acute cadmium intoxication, just as a reduction in the MDA concentration in cases when the experimental animals were given a glutathione supplement implies decreased lipid peroxidation.

Poisoning by a sub-lethal dose of lead causes a slightly greater production of MDA in the liver in comparison to sub-lethal doses cadmium poisoning, which correlates with the results of the roles of heavy metals in lipid peroxidation.[33] In the case of kidneys, cadmium intoxication causes a slightly greater production of MDA in comparison to lead intoxication, probably due to the difference in their metabolism and rate of excretion.

The kidneys represent the primary organs which enable the extraction of GSH from the peripheral bloodstream.[34,35] GSH is actively synthesized and secreted in the kidneys. In addition to kidneys, other tissue types, for example the lungs and the epithelium of the intestinal tract, participate in the re-synthesis and inter organ flow of GSH. In the case of intense oxidative stress, GSH extraction from the liver into the peripheral bloodstream is increased.[36] In this way the remaining organs have easier access to GSH.

GSH forms low solubility mercaptides with heavy metal ions *via* its –SH group. However, GSH can make complexes of heavy metal ions. The stability of the complex which GSH may create with heavy metal ions (Pb^{2+} and Cd^{2+} in this case) depends on the size of the ions, the acid–base characteristics of the ions as well as the affinity of the –SH group for these ions. Adding GSH, one day after the exposure of experimental animals to these metals during the experiment, partially reduced the effect of their toxic influence.

Metals with a stable valence (Cd and Pb) express an indirect pro-oxidant effect, as do metals with variable valence (Cu, Fe, Hg and Cr), by interacting with bio-elements, *i.e.*, by reacting with metals in the active centre of an enzyme, through interference in the same ion channels and the disturbance of the intercellular homeostasis of ions, thus contributing to the induction and propagation

of the lipid peroxidation process.[37] The bonding with biomolecules is realised *via* the –O, –N or –S donor atoms of these bio-ligands, so that their effective concentration may be reduced together with their availability for interaction under the physiological conditions of the functioning of the human body.

The measured content of metal in the liver was higher in the case of animals which one day after poisoning received the supplement (GSH) which, by bonding with the metal, contributes to its retention in the tissue. In the liver and kidney homogenates, the presence of lead was registered, along with a minor reduction in the concentration of lead in the tissue samples in the presence of the supplement. Even though they are present in the tissue samples, these toxic metals have a lower toxic effect, as measured *via* the MDA concentration levels, which is the result of the partial blocking of toxic metals achieved through bonding through the active centres. GSH as a ligand with a potentially large number of donor atoms (–O from the –COOH group, –N from the $–NH_2$ group, –S from the –SH group) can initiate various interactions with metal ions (Me^{2+}), depending on the radius of the ions of these metals ($r_{Pb^{2+}} > r_{Cd^{2+}}$).[38,39] The donor atoms of GSH that could potentially interact with Me^{2+} are represented in Fig. 4.

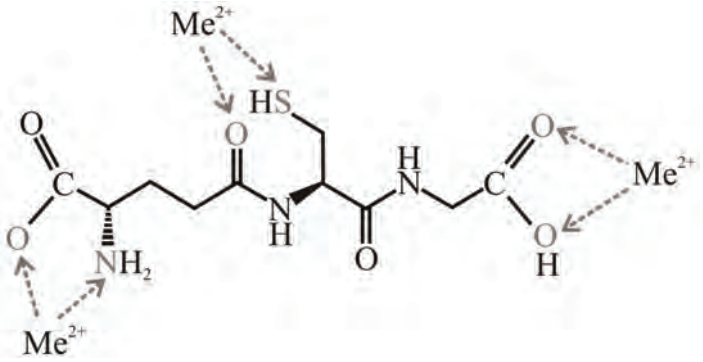

Fig. 4. Donor GSH atoms for the potential interactions with Me^{2+}.

According to the obtained results, GSH more effectively blocks, or reduces, the toxic effect of Cd^{2+}. The results indicate that in the presence of GSH, larger amounts of these metals are accumulated in the analyzed tissue samples, but the negative effect of their presence is lower than in the absence of GSH.

CONCLUSIONS

This study, performed on a model system involving experimental animals intoxicated by sub-lethal doses of Cd and Pb showed that the effects of intoxication, apart from measurement of the toxic metal contents in biological fluids, may also be monitored *via* an indicator of the level of lipid peroxidation, *i.e.*, MDA concentrations. In situations where intoxication *via* these metals occurs,

GSH has a protective role during and also following intoxication. This supplement contains a free –SH group that forms stable associations with the ions of these metals, thus blocking the heavy metals and reducing their toxic effects. The intake of food rich with GSH supplement and of products of a similar structure may have a preventive effect, and significantly reduce the toxic influence of these metals.

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И З В О Д

ЗАШТИТНА УЛОГА ГЛУТАТИОНА У ИНТОКСИКАЦИЈИ КАДМИЈУМОМ И ОЛОВОМ. ПРАЋЕЊЕ ПРЕКО САДРЖАЈА МАЛОНДИАЛДЕХИДА

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Тешки метали (Cd и Pb) представљају озбиљан еколошки проблем због њихове растворљивости, покретљивости и акумулацији у земљишту. Изложеност тешким металима, кадмијуму и олову, може бити из различитих извора (вода за пиће, храна, ваздух, итд.), а у организам човека могу доспети преко респираторног и дигестивног система. У раду је испитиван негативан ефекат интоксикације сублеталним дозама олова или кадмијума преко мерења садржаја у хомогенатима јетре и бубрега малондиалдехида, који настаје у процесу липидне пероксидације. Студија је урађена на модел систему експерименталних животиња (албино пацови Wistar соја). Садржај малондиалдехида је одређиван спектрофотометријски. Резултати ове студије су показали да у условима интоксикације овим металима долази до вишеструког повећања садржаја малондиалдехида. Овај ефекат је изразитији у јетри. Глутатион, који се у физиолошким условима синтетише у организму, као потенцијално полидентатни лиганд са већим бројем донор атома може бити суплемент који испољава протективну улогу у случајевима интоксикације овим металима без обзира на акумулацију истих у анализираним ткивима.

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