Our study was designed to evaluate renal function changes regarding concentration on some minerals in kidney (Cu, Zn, Mn, Cr, Pb, Al) and some renal biochemical parameters (uric acid, creatinine, urea) after administration of manganese overdoses to Wistar strain rats. For this experimental research we worked with three groups such as: one control group – C and two experimental groups – E1 and E2. After several days of accommodation we started to administrated MnCl2 solutions in two different doses: twice of recommended daily intake (RDIx2) and fourth of RDI for E1 and E2 group, and for the C group we administrated drinking water. The administration was made in the day 4th and 7th of experiment and we analyzed the concentration of uric acid, creatinine and blood urea nitrogen in serum, and also, the concentration of Mn, Zn, Cu, Al, Pb, and Cr from renal tissue. The results showed that the excretion of uric acid, creatinine, and blood urea nitrogen increased for both E2 and E2 groups compared to C group. Also – in experimental groups – concentration of Mn, Zn, Cr, Pb, and Al increased compared to control group, but concentration of Cu decreased to both experimental groups compared to control group. This experiment demonstrates that high doses of manganese increase the excretion of uric acid, creatinine, and urea through kidney, and the renal system will concentrate some bioelements and some potential toxic elements.

Key words: manganese overdoses, kidney, rats

For living organisms, manganese is an essential trace element, being necessary for activity of nervous system, bones, milk production and reproduction functions, but is found mostly in bones, liver, kidney and pancreas. In humans and animals, manganese is involved in lipid, carbohydrate, protein metabolism, and calcium absorption. Also, manganese acts as enzymatic cofactor (for example for arginase, cholinesterase, phosphoglucomutase, pyruvat carboxylase, and also some phosphates, peptidases and glycosyltransferases) and is direct component of manganese superoxide dismutase (MnSOD) – mitochondrial antioxidant that protects the cellular membranes of free radical damage [1-3].

Free radicals occur naturally in the organism but can damage the cell membranes and DNA. Recent study demonstrates that inactivation of manganese superoxide dismutase (MnSOD) is associated with renal disorders; although the mechanism is not very clear [4].

Almost half of the total amount of manganese from organism, about 15-20mg, is distributed to bones and the rest quantity is distributed to different organs such as: liver, pancreas, kidney, pituitary and adrenal glands [5,6].

Manganese availability is very strong lead to the chemical form of ingested manganese. Thus, in human or animal body, manganese can be found as different manganese salts, such as: manganese sulfate or manganese gluconate; or manganese chelates as aspartate, fumarate, malate, succinate, citrate, etc. The combination of manganese with carbon is characteristic for organic pesticides (ex.: mane, mancozeb) that can contaminate the food stuffs for humans and the feed for animals [7].

Manganese is essential micronutrient and it is necessary to get manganese from food and feed for a good balanced nutrition. Water, nuts, whole grains, instant coffee, powder cocoa, tea, meat and other products are very good sources for manganese.

Too high concentration of manganese in water, soil or environment may occur due to manufacture, or using insecticides and pesticides in technology of food and feed production. Overdoses of manganese intake may cause damage of some organs such as: brain, liver, and kidney. Usually, humans and animals can not overtake manganese from food or feed stuffs, and usually overdoses come from nutritional supplements or drinking water with high manganese concentration. The bioavailability of manganese is greater in supplements and water then in food or feed products.

Experimental part

For experimental work our study was design on laboratory animals: adult rats from Rattus norvegicus breed, line Wistar, of different sexes, but with similar somatic characteristics and every group had 10 animals each. The technique used for administration the manganese overdoses was the gavage of a manganese chloride solution in two different concentrations.

Animals were maintained in good physiological conditions, according with Romanian and European Union laws (Low 471/2002, Regulation 37/2002) concerning animal protection in scientific researches [8,9]. At all three groups in the fourth day of experiment, we have administered first dose of solutions, then in the seventh day the second one, and the experimental animals were treated the same as experimental animals, but we have administrated drinking water as gavage.

Before gavage administration, a procedure with ketamine intramuscular injection was made to animals (Calypsol 10ml/500mg ketamine chloride, produced by “Gedeon Richter Ltd.”, Budapest, Hungary) for anesthesia.
The references for metal concentration of administrated solution were RDI – Recommended Daily Intake for manganese. From manganese chloride, we had administered two concentrations, double RDI (RDIx2 for E1) and quadruple RDI (RDIx4 for E2) concentration for manganese. As a reference for manganese concentrations of metals, we had the recommendations of Expert Group on Vitamins and Minerals Meeting - Nutrition, which are: 0.171-0.214 mg/kg b.w. of manganese, daily. So, for the first administered dose as RDIx2 of Mn (E1 group), we prepared a 1.550mmol/L MnCl₂ solution, and for the second administrated dose RDIx4 of Mn (E₂ group), we prepared a 3.110mmol/L MnCl₂ solution [10].

After gavage procedure, animals were behavioral checked and no problems were registered. At the finish of experiment – in the fourteenth day, after anesthesia with ketamine, we collect the blood samples and after dissection, we sampled the kidney. Sampling was made with sterile surgical instruments, and organs were stored in clean glass containers in freezer.

Concentration of uric acid (URIC), creatinine (CREA) and blood urea nitrogen (BUN) from blood serum was performed with automatic clinical biochemistry equipment, and the results were expressed in mg/dl serum.

Digestion of kidney was made in a Millestone Microwave System with a special program for samples with fast exothermic reactions (containing a large amount of organic matter). After wet digestion with concentrated nitric acid (Merck) and H₂O₂ solution, sample solution was transferred into a volumetric flask and then was diluted to volume with double deionized water. The equipment used for metal determination in solutions was atomic absorption spectrometer (AAS), produced by Perkin-Elmer, with Zeeman effect for background correction and transversal healing of graphite tube, with the determination in air-acetylene flame (AA), or by electro thermal atomization – depending on the element. We used appropriate ionization control substances for flame and matrix modifiers in graphite tube.

For instrument calibration, we used standard single element solutions of 1000mg/L, produced by Merck, making dilutions for calibration standards, to obtain a calibration curve in linear domain. The calibration curve control was made with a multielement standard solution (Merck). The results were obtained in μg/L in solution, and reported after calculations in μg/g w.t., considering the initial weight of tissue and the volume of volumetric flask used (50 mL).

Statistical data were obtained using descriptive statistics.

Results and discussions

We evaluated the concentration of some blood serum parameters such as: uric acid, creatinine, and blood urea nitrogen – that gives us information about the kidney status. Also, we evaluated the concentration of some bioelements (Mn, Zn, and Cu), and some potential toxic elements (Al, Pb, and Cr) from kidney tissue in order to see the concentration of these elements in the organ that is responsible with the excretion of elements in high doses.

Distribution of blood serum biochemical parameters at the end of manganese administration in high doses – animal experiment, was presented in figure 1.

After evaluation of the results we can easily see that overdoses of manganese increased the concentration of uric acid, creatinine, and blood urea nitrogen to experimental groups compared to control group. Thus, uric acid increased to 3.34mg/dl for E₁, and 3.5mg/dl to E₂ group compared to C group. Also, creatinine concentration in serum increased to 0.70mg/dl for E₁, and 0.75mg/dl for E₂ group compared to C group. Blood urea nitrogen was higher to experimental animals: 38.8md/dl for E₁, and 37mg/dl for E₂ compared to control animals. This process showed that after administration of manganese in high doses, protidic metabolism was influenced and the renal excretion of metabolites was accelerated.

Manganese is a metal element that is involved to protidic metabolic pathways and is part of the structure of different enzymes with important role to antioxidant protection for organism [10]. One of these enzymes is the superoxide dismutase bounded with manganese (Mn²⁺) in found in the mitocondria. Three histidine side-chains, aspartate side-chain and hydroxy ligand are the ligands of manganese ions, when the manganese oxidation state depends on Mn³⁺ or Mn⁴⁺.

The optimal concentration of manganese in the tissues (manganese homeostasis) is possible mainly by absorption and bile or/and intestinal excretion. Only 15% to 30% from the ingested manganese is used by organism, so manganese absorption is low. The absorption of manganese is influenced by the presence of other metal elements such as: zinc, copper, iron and calcium, and also by the presence of lecithin, soy protein, chemical form of intake manganese and alcohol. Manganese is bond to a transporter globulin protein – transmangamin – and is vehicle thought blood to different tissues where is needed [11].

The concentration of zinc, manganese, copper, aluminum, lead, and chromium was calculated after we performed the atomic absorption spectroscopy and the results were graphical represented. Figure 2 and 3 represents the distribution of Mn, Zn, and Cu (fig. 2) and Al, Pb, and Cr (fig. 3) in rats’ kidney after gavage intake of manganese in excess.

Concentration of manganese and zinc was higher in kidney for experimental groups compared to control group, after administration of manganese in overdoses. Manganese acts as a synergic compound for excretion of zinc in renal system, dislocates the zinc deposits from different tissues and transport it to the kidney for excretion. Thus, zinc concentration in kidney rise to 21.01 μg/g for E₁ (with 6.09%) and to 21.19μg/g for E₂ (with 7.89%), compared to control group (19.64 μg/g) [10].

Also, manganese is higher in kidney tissue after overdoses of manganese for a short period of time. Concentration of manganese was 0.79 μg/g for E₁ (16.17%) and 0.87 μg/g for E₂ (27.94%) compared to 0.68 μg/g for C group.

Copper concentration decreased in kidney after overdoses of manganese for a short period of time. Thus, the quantity of copper in kidney decreased to 3.41μg/g for E₁, (with 31.8%) and to 2.69μg/g for E₂ (46.2%) compared to 5μg/g for C group. In fact, excess of
manganese intake has antagonist effect with copper in renal tissues (kidney).

Also, Quintanar and his research team in 2008 demonstrated that excess of manganese intake lead to iron and copper deficiency and the absorption of manganese would interfere with calcium and phosphorus homeostasis [11].

Having in view our experimental results we can say, based also on other research studies, that variations of concentration of manganese and copper in kidney tissues are owned to enzymatic changes (catalase and superoxide dismutase) that are responsible for antioxidant protection against cellular oxidation.

For aluminum, lead and chromium, overdoses of manganese intake had synergistic effect in our experiment. Manganese overdoses administrated to rats presents significant variation for lead distribution in kidney, and easy increased the quantum of aluminum and chromium in rats' renal tissue.

Aluminum concentration in kidney had high variation after manganese administration in excess, thus, the distribution of aluminum in kidney showed an increased with 18.7% for E1 and 37.23% for E2 Wistar rats compared to control group of animals.

Distribution of lead registered the significant variation after excess of manganese as gavage intake, so after administration of Mn the concentration of Pb was 1.8 times higher for E1 and 3 times higher for E2 group compared to control group. This can explained a very strong synergistic effect between excess of manganese and lead, thus an overdoses of manganese can dislocate the lead from other tissues and concentrate the lead to kidney for excretion. Our experiment can be very useful for cases of intoxication with lead, and can be a solution of higher renal excretion of lead from different tissues, and decreases the concentration of lead from intoxicated organism.

Conclusions
Manganese excess for a short period of time modifies the biochemical homeostasis of some serum biochemical metabolites (uric acid, creatinine, blood urea nitrogen) and also some mineral elements in rat's renal tissues (kidney).

High doses of manganese intake for a short period of time increased the concentration on uric acid, creatinine and blood urea nitrogen that characterized the renal excretion function.

Concentration of analyzed trace elements from blood serum (Mn, Cu, Zn) presented modifications after overdoses on manganese. Thus, manganese and zinc were higher for experimental groups with high intake of manganese, but copper concentration decreased in kidney tissue.

Manganese overdosed intake has synergistic effects for manganese and zinc in kidney and has antagonistic effect for copper in renal tissue.

After excess of manganese intake, in kidney, lead concentration increases very much for experimental animals compared to control animals. Also, aluminum and chromium concentration in kidney is higher after gavage of manganese compared to control Wistar rats from control group.

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