Rabbits *in vivo* Experiments and Hematological Evaluation after Iron Toxicity

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Abstract
Iron is essential elements for human, animals and plant organism. The depletion of organism in iron leads to modification in the concentration of hemoglobin which is responsible for oxygen transportation. Also, overload of iron represents a possible intoxication that can affect negatively the liver tissue and then all cells. Our experiments was conducted on two groups (control and experimental) of rabbits – German Lop Eared Rabbits breed, and we administrated ferrous gluconate (10mg/kg body weight) to the experimental group, two times. Also, during the experiment the rabbits had a special diet with plants from garden administrated fresh. The plants have very good antioxidant properties, and can prevent the iron intoxication and also participate intense to detoxification of the liver after iron overload. Hematological analysis were preformed for red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet and leukocyte evaluation. The results demonstrated a very good protective action of the diet plants, meaning that the hematological parameters were not out of normal range after iron overload.

Key words: rabbit, iron, protective diet

Introduction
Iron is an essential element found all over in living organisms and environment; is a transitional metal, and is the fourth most representative metal in the Earth's crust. Biochemical properties of iron are various being involved in many oxido-reduction processes – by changing the oxidation states from Fe^{2+} to Fe^{3+} or reverse. Iron is present in different essential compounds in organism, but most known is the prophyrinic compound that contain ferric ion – hemoglobin. Also, many enzymes activity are depending on the presence of iron. Main functions of iron in living organism are: growth-promoting capacity, transportation of oxygen, in energetic metabolism: conversion of glucose from blood in energy, DNA synthesis, and more others (Seo et al., 2014). The lost of iron from organism has to take in view the urination, sweating, defecation, old skin cells exfoliating, and bleeding (menstruation).
The quantum of iron from organism is very important, and the equilibrum between the intake and excresion has to be very well managed by living organism because also the excess and deficiency (microcytic or hypochromic or severe anemia; primary and secondary hemochromatosis) can have severe consequences (Ludwiczek et al., 2005; Chua et al., 2007; Naigamwalla et al., 2012). However, in case of insufficient iron, the concentration of hemoglobin is also reduces that lead to a decrease of oxygen transport with consequences

such as fatigue, vertigo, or loss of energy in case of athletes (Mettler and Zimmermann, 2010).
Iron bioavailability is depending by dietary composition, and very important by the chemical form of the iron salt (Hurrel and Egli, 2010).
More of 90% of necessary iron has to come from endogenous sources because of the metabolic pathway of iron in concordance with the red cells. The necessary iron for organism has to count the losses of iron and the needs of iron for growth and has to be provided by a proper diet. Also, the dietary characteristic influences the iron bioavailability as non-heme-iron (present in plans and animals) and heme-iron (from hemoglobin and myoglobin). Between these two iron forms, the heme-iron is better absorbed compared to non-heme-iron. However, there are some other factors that influence the iron absorption, such as the presence of some chemical compounds that bound the iron and made it insoluble, and finally is excreted by feces. These compounds are usually acids like phytate (myo-inositol hexakisphosphate) well presented in some plants with green leaves and some grains (Gupta et al., 2015; Hurrell and Egli, 2010; Sotelo et al., 2010).

Experimental

For this experimental data we used German Lop Eared Rabbits breed, as two groups of animals: one control group and one experimental group (Dronca, 2007). Each group of rabbits was formed by five animals. The animals where in a well suited placed, without to be stressed, and had well physiological conditions according with specific laws concerning animal protection in scientific researches (Romanian Law nr 205/2004; Directive 86/609/EEC from November 24, 1986 for Protection of Vertebrate Animals Used for Experimental and Other Sciences Purpose and Directive 2010/63/EU for Protection of Animals Used for Experimental and Other Scientific Purposes). The environment was well ventilated and the diet was establish in order to bring a toxicity protection against iron overload (Hazra et al, 2012). Dietary feed including organic plants from garden that provided a protective and a pro-active antioxidant protection (well-ahead of the addition of the toxicity of iron administration). The rabbits had in their diet one time a week oat (*Avena sativa*), dry alfalfa (*Medicago sativa*), carrots (*Daucus carota subsp sativus*), clover (*Trifolium*), garden parsley (*Petroselinum crispum*), leek (*Allium ampeloprasum*), chives (*Allium tuberosum*), coriander (*Coriandrum sativum*), and fenugreek (*Trigonella foenum-graecum*). These plants were organic plants, cultivated in our garden, and were fresh administrated in the rabbit's diet.

We choose to administrate iron as Fe(II)-gluconate hydrated (Fluka) because it was commercially available, is water soluble and the iron from this product has good bioavailability in animal organism. We administrated iron such as ferrous gluconate (C12H26FeO16) as a solution that provided 10mg Fe^{2+} / body weight for every rabbit from the experimental group, twice times during our experiment, with a pause between administrations of 7 days. The animals from control and experimental group where introduced into the experiment right after the weaning period (after five weeks of life), the weight of the rabbits was between 580g and 800g (weight mean was 703g and SD was 89.19) and the experiment was conducted during 43 days during the summer time. Blood samples were collected for hematological determinations. By tube inversions we ensure mixing of anticoagulant K3EDTA with blood to prevent clotting. Sampling the blood from rabbits had in view the good practice guidelines (Waynforth et al., 1998). Complete blood count was

analyzed in the authorized bioclinical laboratory in Timisoara, with Impedance Analysis System, Flow Cytometry method.

Results and discussion

Our experiment wants to have in consideration the evaluation of some hematological parameters after iron overload in experimental rabbits compared to the control group. In the figure 1 we present the results of red blood cells (RBC or erythrocytes), hemoglobin (HB), hematocrit (HC), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), platelet (PL) and leukocyte (WBC – white blood cells).

The hemoglobin (HB) is the protein that participate to oxygen carrying in blood. Hematocrite (HC) represents the percent (%) of the red blood cells from the total blood volume. The red blood cells (RBC) indicates more informations about the heath status, and these tests are: mean corpuscular volume (MCV) that represents an avarage size of a single red blood cell; mean corpuscular hemoglobin (MCH) that presents the avarage of hemoglobin inside a red blood cell; mean corpuscular hemoglobin concentration (MCHC) represents a calculation of an avarage hemoglobin concentration inside the red blood cells; red cell distribution width (RDW) represent a calculation of RBCs size variation.

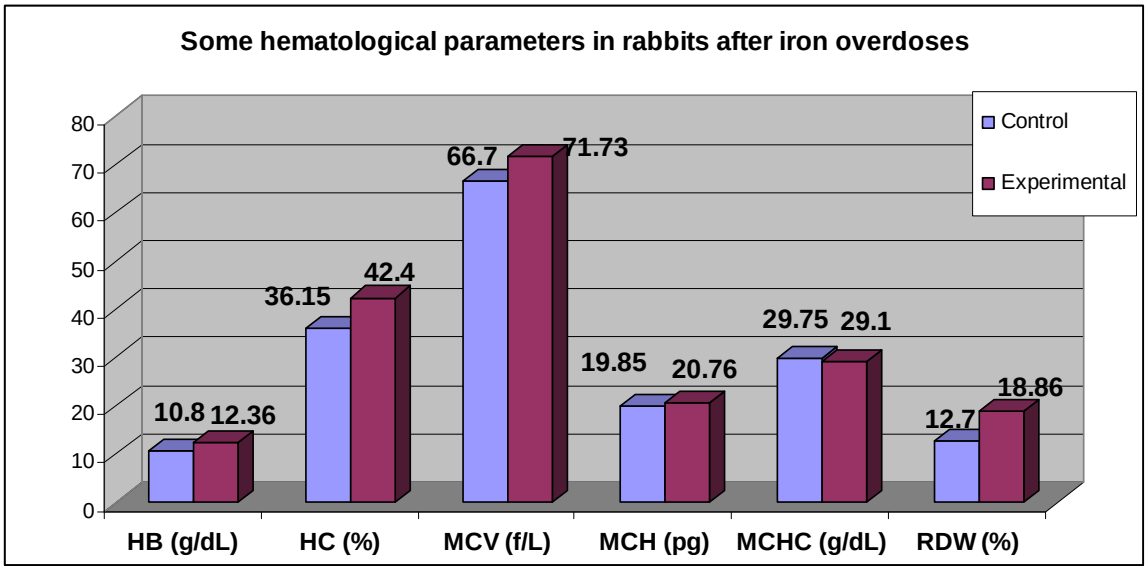

Figure 1. Variation of some blood hematological parameters of rabbits

After iron glconate administration for a very short time, the rabbits accummulates more iron compated to the control group, but compared to the normal range of these parameters the values from experiment are in normal range. This can be explained that all animals where feeded wit a special detoxifiant diet, based on different plants with protective action against iron intoxication.

Evaluation of reb blood cells (RBC) represent the total number of red blood cells (also named erythrocytes) presented in total blood. The platelet evaluation presents the cell fragments that play a vital role for normal blood clotting and the platelet count is the plateles number from blood; mean platelet volume (MPV) is more often raported together with reticulocyte count (CBC) and with platelet distribution width (PDW) – that reflects the uniformity of plateles in size.

Figure 2 presents the variation of red blood cells (RBC) and platelets in rabbits for control group and experimental group – after administration of moderate excess or iron.

Concentration of RBC and platelets is easy increased in the experimental rabbits that is normal because of the total iron intake in a short time.

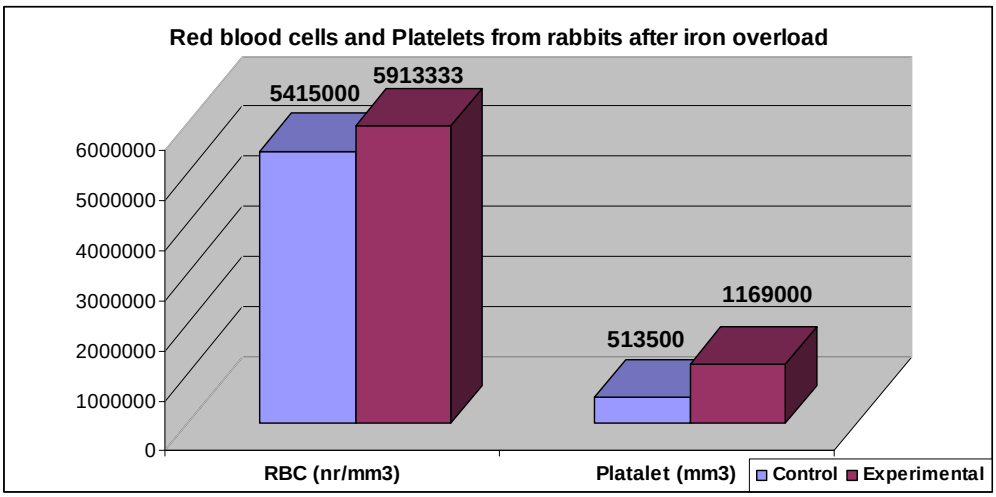

Figure 2. RBC and plateles

Evaluated of WBC – total number of white blood cells, present a respons of the organism against infection and cancer and also play a very important role in allergic reactions and inflammation. White blood cells differenties various types of white blood cells, such as: neutrophils, lymphocytes, monocytes, eosinophils, and basophiles.

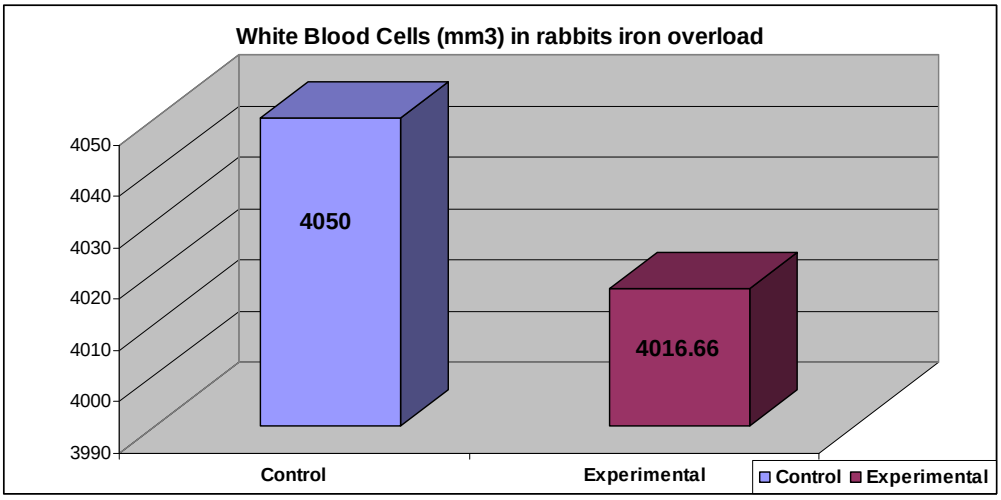

Figure 3. WBC after iron overload in rabbits

White blood cells number is higher in control group compared to experimental group, but both values are in normal range for rabbits. This explains that the immunity is not affected by administration of iron overload for a very short time or another explanation could be link to the diet that could protect the body from an infection of low immunity.

Conclusion

Iron is an element essential for a good heath status, but out of the normal range can affect the immunity, the resistance to effort, can lead to anemia, or in overload case can affect the liver tissue or all the cells integrity.

Diet based on different plants administrated fresh, can prevent and can counteract the moderate toxicity of administration of iron in high concentration for a short time to the

rabbits. Iron overload can not affect negatively the heath status to the rabbits if the diet is conducted to assure a protective action against iron toxicity.
Hematological determination after iron overload for a short time to the rabbits feed with a protective fresh diet, does not affect the normal values for red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet and leukocyte evaluation.

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