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THE INFLUENCE OF POLYPHENOLS FROM NETTLE EXTRACT ON IRON METABOLISM AND SOME MICROELEMENTS THAT PARTICIPATE IN MAINTAINING IRON HOMEOSTASIS

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Abstract

Iron is integrated into metabolic processes, cell growth and proliferation, while also being an essential cofactor of the mitochondrial respiratory chain, the Krebs cycle and DNA synthesis. Maintaining iron homeostasis in the body has always been in the sights of researchers at different levels of organization and composition of living organisms, its role can be determined by the biological functions of some proteins that contain this biometal, such as hemoglobin and myoglobin, enzymes involved in biological oxidation processes, enzymes that neutralize ROS and maintain redox balance in the body. At the same time, the excess of iron is associated with cytotoxic effects, which are caused by the peculiarities of iron, as a metal with variable valence, to trigger chain reactions of free radical formation, which lead to lipid peroxidation (LPO) of biological membranes, toxic damage to proteins and nucleic acids. Therefore, the content of this microelement is strictly regulated, which allows us to talk about iron homeostasis. The purpose of this paper is to familiarize with new scientific results of iron homeostasis under the influence of polyphenols extract from nettle.

Key words*: antioxidants, iron homeostasis, iron overload, oxidative stress, plant extracts.*

INTRODUCTION

Free radicals can be produced due to the influence of environmental pollutants, metal ions, radiation, etc. (Alkadi, 2020). As a result of ROS production, oxidative damage to biomacromolecules occurs, which can represent a suitable environment for disrupting the metabolism of energetic and detoxifying substances at the membrane and cell level (Gasmi et al., 2021). Endogenous defense mechanisms of living organisms cannot ensure complete prevention of damage caused by ROS (Gasmi et al., 2021). Natural antioxidants, as effective removers of free radicals, are indispensable in the prevention and treatment of many disorders (Alkadi, 2020). The concept of homeostasis currently plays an important role in the analysis of vital processes at different levels of biological systems. The homeostatic properties of the whole organism are the result of the simultaneous action of many complexly organized regulatory mechanisms, among which one of the important places is occupied by autonomic regulation, which ensures the constancy of metabolic and energy levels in the body, individual organs and tissues (Baevskii et al., 1984).

Iron is essential for oxygen transport, erythropoiesis, and plays an important role in many biological processes, in particular, such as the synthesis of deoxyribonucleic acids (DNA, RNA), amino acids, collagen, steroids, various neurotransmitters and proteins involved in electrolyte transport for mitochondrial respiration, cell proliferation, differentiation and regulation of gene expression, as well as in the functioning of the immune system, detoxification processes and metabolism of pharmacological remedies (Yiannikourides & Latunde-Dada, 2019). Iron is not synthesized in the human and animal organism, and its homeostasis is generally controlled by dietary

iron intake, intestinal absorption and its recycling mechanisms (Moustarah & Mohiuddin, 2022).

Iron deficiency can be caused by physiological, pathological, environmental, drug, genetic or erythropoietic reasons (WHO, 2020).

Excessive amounts of iron can cause toxicity and cell death through free radical formation and lipid peroxidation; therefore, iron homeostasis is strictly regulated (Bresgen & Eckl, 2015). Iron is mainly used as a component of heme in erythrocytes for oxygen transport, while relatively smaller amounts are present in muscle in the form of heme myoglobin and in the liver in the form of ferritin (Kohgo et al., 2008). In most other tissues in the body, iron serves as a component of protein cofactors in the form of iron-sulfur (Fe-S) clusters and heme, which are involved in the electron transport chain to produce ATP (Beinert et al., 1997). In this process, Fe^{2+} and Fe^{3+} serve as electron donors and acceptors, respectively. Basically, iron plays an essential role in maintaining adequate ATP reserves during oxidative phosphorylation in the mitochondrial internal membrane (Oexle et al., 1999).

The regulation of iron homeostasis is a complex multicomponent process involving a cascade of regulatory proteins. Due to the significant oxidative activity of iron and the ability to form chelate complexes with many compounds, its amount in the body is strictly limited and there are no physiological ways to naturally remove excess iron from the body. Iron levels can directly influence the activity of iron regulatory proteins in addition, with modulatory stimuli being nitric oxide, hypoxia, oxidative stress and erythropoiesis activity (Rumeantzev & Tokareva, 2004).

Disturbance of iron homeostasis is associated with various pathologies. Iron deficiency, resulting from impaired absorption or bioavailability of the metal, causes anemia. Excess iron leads to its deposition in tissues and becomes the pathophysiological basis of many pathologies (Yiannikourides & Latunde-Dada, 2019). High iron levels lead to the formation of free iron (II), which can cause oxidative stress and cell death through lipid peroxidation, known as ferroptosis (Rumeantzev & Tokareva, 2004).

Thus, both iron deficiency and iron overload have catastrophic consequences for the body, and the content of this microelement is strictly regulated.

The purpose of this research was to determine the influence of polyphenols from nettle on the metabolism of iron and other microelements, which participate in this process, namely in maintaining iron homeostasis. The study of iron homeostasis is of great interest due to the need to find new approaches to correcting the severe consequences of iron deficiency and excess in the body by including biologically active substances in the food ration.

MATERIALS AND METHODS

The purpose of this research conducted in our laboratory was to determine the influence of polyphenols from nettle extract on the iron homeostasis in the blood serum of roosters. Ten roosters were used for the research, which were divided into two groups: one control and the other experimental. The roosters in the control group were administered 1 ml of saline per os to each animal using the special dispenser for administering medicinal remedies to animals. The experimental group was administered polyphenol extract from nettle (urtica dioica), in a volume of 1 ml, with a total polyphenol content of 33.2 g/100 ml gallic acid equivalent (GAE).

Calorimetric (photometric) methods were used to determine chemical elements. The amount of iron was determined by the calorimetric method. The principle of the method consists in releasing iron from iron-binding proteins in blood serum and reducing it by the action of an acidic working solution (pH 1.6-1.7), which contains the reducing agent hydroxylamine and detergents. Then, after the addition of a chromogenic ligand (ferrozine) and a buffer, a stable, colored iron-ferrozine complex is formed, the optical density of which is measured at 570 nm.

The determination of zinc was based on the principle of the formation of a colored zinc complex with a special reagent (for example, Br-PPAS), the color intensity of which is proportional to the concentration of zinc, which is measured photometrically (endpoint).

The calorimetric (photocolorimetric) method for determining ceruloplasmin (CP) in blood serum is based on measuring its enzymatic activity (oxidase function). Ceruloplasmin is an enzyme that contains copper and catalyzes the oxidation of various polyamines, most commonly p-phenylenediamine. The principle of the method consists in the oxidation of the substrate, where, in the presence of serum ceruloplasmin, p-phenylenediamine is oxidized to form a colored compound (usually pink or brown). The color intensity of the solution formed during the reaction is directly proportional to the concentration and activity of ceruloplasmin. The optical density of the solution was measured using a photoelectrocolorimeter or a spectrophotometer.

Magnesium was also determined by the calorimetric (photometric) method. This method is based on measuring the intensity of the color formed during the reaction of magnesium ions with specific reagents; the color intensity of a solution is directly proportional to the concentration of magnesium in it (Beer-Lambert law).

The colorimetric method was used to determine phosphorus in blood serum (phosphomolybdate reaction). This method is based on the reaction of phosphates with ammonium molybdate in an acidic medium. The principle is that phosphates react with ammonium molybdate to form phosphomolybdic acid. This acid is reduced (usually with ascorbic acid or other reducing agents) to a blue compound, the intensity of which is directly proportional to the concentration of phosphorus.

The duration of administration of the preparations was for a period of 60 days.

The animals were maintained in the vivarium of the Institute of Physiology and Sanocreatology of the Moldova State University. The conditions of animal maintenance correspond to the sanitary and veterinary requirements for the maintenance of laboratory animals.

Biochemical investigations were carried out in the scientific-practical biochemistry laboratory of the "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova.

Blood samples from birds were collected from the ulnar cutaneous vein (wing vein), in compliance with all sanitary and veterinary requirements for collecting blood samples from laboratory animals.

The statistical analysis of the obtained data was carried out using the Student's t-test (Novikov, 1995).

RESULTS AND DISCUSSIONS

In these studies, the state of hematological indices in the general analysis of blood, which are dependent on iron, was evaluated after the administration of hydroalcoholic extract from nettle. The research results are presented in Table 1.

Table 1. Hematological indices of reproductive roosters included in experiments to study the action of polyphenols from nettle (*Urtica dioica* L.) on the reproductive system and the organism as a whole

Groups	Hgb (g/dl)	RBC, $10^{12/l}$	MCV (fl)	MCHC (g/dl)	MCH (pg)
Control	138.6±3.51	3.22±0.33	132.4±0.69	30.7±0.35	38.9±0.65
Experimental	151.0±2.00*	3.64±0.32	130.0±1.12	30.5±0.30	39.6±0.16

*The differences are statistically true between the experimental and control group ($P<0.05$)

Note: Hgb – hemoglobin concentration; RBC – absolute number of erythrocytes in the blood (red blood cells); MCV – mean corpuscular volume; MCHC – mean corpuscular hemoglobin concentration; MCH – mean corpuscular hemoglobin; fl – femtoliter; pg – pictograms.

From the results obtained, we observe that the amount of hemoglobin in the experimental group indicates a value of 151.0±2.0, compared to the control group with a value of 138.6±3.51 g/dl, which proves us about a stimulating effect of the polyphenol extract from nettle (*Urtica dioica* L.) and its influence on iron metabolism in living organisms.

As a result of the analysis of the specialized literature, it turns out that hemoglobin (Hb) is a molecule that is functionally linked to aerobic organisms and participates in the use of oxygen from the atmosphere, which it delivers to cells

and tissues. It is thanks to hemoglobin, the cardiovascular system is supplied with oxygen, through which are maintained cellular homeostasis and signaling the protective function of the cell. These roles are particularly highlighted in the binding of Hb to the gaseous molecules of carbon dioxide (CO_2), nitric oxide (NO) and carbon monoxide (CO), also serving indirectly or directly as sources of these signaling molecules.

In erythrocytes, $NO^{\bullet}$ will react with HbO_2 to form MetHb and nitrate, as well as with $O^{2\bullet}$- to form the strong oxidizing agent $ONOO^-$ (Stuehr & Haque, 2019).

The end result of oxidative damage to erythrocytes is hemolysis, loss of membrane integrity and release of hemoglobin and other intracellular proteins. Free hemoglobin is particularly toxic, and this is obvious that it can cause damage of erythrocytes (Schaer et al., 2013).

Polyunsaturated fatty acids (PUFAs), 18% of the total fatty acids in RBCs (Pacetti et al., 2016), are the lipid components that are more sensitive to oxidation in a series of reactions that trigger lipoperoxidation.

The antioxidant defense allows normal erythrocytes to survive 120 days in circulation. The main protection against oxidative damage is provided by various enzyme systems, such as superoxide dismutase, catalase, glutathione, etc.

MCV is a value that measures the average size of red blood cells. In states of excessive oxidative stress (EOS) in different individuals, the antioxidant capacity of erythrocytes is decreased (Maritim et al., 2003). From the results of the conducted researches we observe a difference in the MCV values, where for the control group a value of 132.4±0.69 fl is indicated, and for the experimental group it represents a value of 130.0±1.12 fl.

The next study of this experiment was devoted to the determination of the mineral antioxidant status in the blood serum of breeding roosters after administration of the polyphenol extract of nettle (*Urtica dioica* L.). The results obtained are presented in Table 2.

Table 2. Mineral antioxidant status in the blood serum of roosters after administration of polyphenol extract of nettle (*Urtica dioica* L.)

Groups	Iron, μM/L	Zinc, μM/L	CP, mg/L	Magnesium, mM/L	Phosphorus, mM/L
Control	12.15±0.31	31.32±0.45	118.17±0.35	0.67±0.03	1.45±0.11
Experimental	14.95±0.73*	39.73±0.89*	131.42±1.62*	0.97±0.06*	1.60±0.11

*The differences are statistically true between the experimental and control group ($P<0.05$)

Approximately 25% of all proteins included in the Protein Data Bank contain metals. Zinc ions are the most prevalent, followed by iron, calcium, manganese and magnesium. The role of transition metals in protein biochemistry is varied. They are part of the active site and actively participate in the formation of protein complexes, such as lipoproteins and nucleoproteins, and also stabilize the structure of proteins with several subunits (Dutta & Bahar, 2010). At the same time, many proteins, under stress conditions, form additional metal-binding centers, including iron.

The most important metal-ligand forms of iron are transferrin, albumin, ferritin, citrate and carnosine.

In the human body, iron metabolism is a strictly regulated process: iron enters the bloodstream from the intestine in the form of a divalent cation Fe(II), which is easily oxidized to Fe(III) and bound to transferrin (80 kDa) (Soldin et al., 2004). Plasma transferrin is the most important physiological source of iron and is synthesized in the liver. Together with ferritin, it binds virtually all circulating iron in the plasma. Under physiological conditions, this chelation maintains low levels of free iron in the bloodstream to prevent the formation of reactive oxygen species and promotes iron transport into cells (Soldin et al., 2004).

Iron-bound transferrin distributes iron to other cells in the body by binding to receptors on cell surfaces and then importing it into the cell through endocytosis. Once in the cytoplasm, iron is transported to various intracellular sites, including mitochondria for the biosynthesis of

heme and ferritin, which in turn serves as an intracellular depot of iron (Ren & Walczyk, 2014). Metabolically inactive iron is stored in ferritin and is in equilibrium with metabolic iron bound to carrier molecules (Ren & Walczyk, 2014).

The ability of albumin to bind iron ions has been repeatedly observed in experiments, although it is believed that this protein does not have specific binding sites for this metal. Serum albumin, known as a low-affinity iron-binding protein, has been proposed as a ligand for the reserve of iron unbound to transferrin, usually present where surplus iron is identified (Rabbani & Ahn, 2019). Albumin carries a net negative charge, with a large number of carboxylic acids on the surface of the molecule, which can form potential iron binding sites (Rabbani & Ahn, 2019).

The involvement of the carboxyl groups of aspartic and glutamic acid residues, as well as the imidazole group of histidine, in the coordination of iron ions was observed. The absence of specific binding sites of iron with albumin is due to ferritin, which is contained in the blood and has a role in the transport and deposition of this metal (Thompson, 2022).

Ferritin is a protein that forms as a complex between ferrous hydroxide and another protein called apoferritin. Ferritin represents the storage form of iron in the body, its concentration highlighting the iron deposits present in the organism of animals, including humans. Ferritin represents a 450 kDa complex that binds iron oxyhydroxide particles (up to 4500 iron atoms). Most ferritins are found inside cells and serve to store iron (Orino & Watanabe, 2019).

Research conducted on the chemical specification of elements in blood plasma suggested the presence of only the trivalent form of iron and indicated that among low molecular weight ligands, iron is present exclusively in the form of a hydroxycitrate complex (Dziuba et al., 2018). However, it has now been established that blood plasma and serum contain two to six types of low molecular weight iron complexes. Citrate, acetate, pyruvate and phosphates are potential low molecular weight ligands of the metal. Considering the affinity of each ligand, researchers have suggested that citrate is the most dominant ligand for unbound iron to transferrin (Dziuba et al., 2018).

Carnosine is a good chelator of iron, copper and zinc ions, eliminates reactive oxygen and nitrogen species and forms complexes with toxic electrophilic α, β-unsaturated aldehydes (Abdelkader et al., 2016). In living organisms, carnosine performs antioxidant and detoxifying functions, neutralizing many potentially harmful substances. It has been proven that it can exert both direct and indirect antioxidant effects. The direct antioxidant action of carnosine is mediated by binding reactive carbonyl compounds, harmful products of fat and carbohydrate oxidation, while its indirect action is mediated by activating the transcription factor Nrf2, which is essential for protecting cells from oxidative stress, metabolic byproducts and toxins.

Thus, the evaluation of the content of iron complexes with high and low molecular weight ligands in vivo is important for a surveillance of metabolism and maintenance at an optimal level the homeostasis of this microelement. In our research, we obtained the following iron values, 12.15±0.31 μM/L for the control group and 14.95±0.73 μM/L for the experimental group.

Zinc is a key modulator of iron absorption and its distribution in tissues. Reducing the level of zinc directly leads to decreased zinc content in the pancreas, which in turn reduces the intestinal absorption of iron by reducing the expression of divalent metal transport and ferroportin. Mechanically, the overall impact of zinc deficiency is associated with the development of iron deficiency caused by a combination of reduced intestinal absorption and reduced mobilization of iron from deposits (Kondaiah et al., 2019). In our research we obtained the following values of zinc, for the control group 31.32±0.45 μM/L, and for the experimental group 39.73±0.89 μM/L.

Ceruloplasmin (CP) also participates in the regulation of iron homeostasis, being an enzyme with an increased copper content, manifesting an increased ferroxidase activity, which is detected under the soluble isoform in plasma or as a membrane-associated isoform in different cell types. The ceruloplasmin-ferroportin system represents the main cellular iron exit pathway in vertebrates and is

responsible for the physiological regulation of cellular iron levels. This enzyme possesses several functions, from copper transport to the oxidation of ferrous iron and biological amines, as well as antioxidant activity by preventing the formation of free radicals in the serum (Floris et al., 2000). In our research, we obtained the following values of ceruloplasmin, for the control group 118.17±0.35 mg/l, and for the experimental group 131.42±1.62 mg/l, which demonstrates about the influence of polyphenols extracted from nettle on the amount of ceruloplasmin, which in turn, through various mechanisms, participates in maintaining iron homeostasis.

Magnesium plays a key role in the regulation of iron homeostasis, influencing its bioavailability, absorption and metabolic processes. The interaction between magnesium and iron is a complex electrolyte balance, in which magnesium can act as a regulator, as it is essential for the proper functioning of hundreds of enzymes, which indirectly support metabolic processes, including iron use.

As discussed in this paper iron can maintain the balance of the normal functioning of the body, but it can also trigger reactions that cause oxidative stress.

CONCLUSIONS

So as can be seen, Iron (Fe) is an essential element for all living organisms, since it is a component of the functional groups of proteins and enzymes that transport oxygen and catalyze energy-generating reactions and metabolic processes. Excess free iron leads to local tissue damage due to increased formation of free radicals. Therefore, the safe range of iron levels in the body is quite narrow and strictly controlled in order to avoid both iron deficiency and iron overload. The research results demonstrated a change in iron values under the influence of polyphenols extracted from *Urtica dioica*, namely 12.15±0.31 μM/L for the control group and 14.95±0.73 μM/L for the experimental group. Changes are also observed in other biochemical indices, which have been studied and are directly at the base of iron metabolism, which contributes to maintaining the homeostasis of this element.

Through the transport proteins of metal ions, such as albumin, ferritin and carnosine, and through the administration of polyphenol extracts with antioxidant effect it is possible to optimize iron metabolism, which will favor the establishment of iron homeostasis, the normal functioning and detoxification of the body's cells.

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