Scientific Papers. Series D. Animal Science. Vol. LXV, No. 2, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF HYDROALCOHOLIC EXTRACT FROM GREEN WALNUT ON CERULOPLASMINE CHANGES IN BLOOD SERUM OF BREEDING ROOSTERS

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Abstract

Ceruloplasmin is a ferment with a high copper content, manifesting an increased ferroxidase activity, which is detected as a soluble isoform in plasma or as a membrane-associated isoform in different cell types. The ceruloplasmin-ferroportin system is the main route of cellular iron exit in vertebrates and is responsible for the physiological regulation of cellular iron levels. Ceruloplasmin is a copper-containing ferroxydase and plays an important role in the ionic state regulation of iron oxidation - Fe^{2+} to Fe^{3+}. As a result, iron is incorporated into transferrin without the formation of toxic iron products. Maintaining the normal transport and metabolism of iron is a function of ceruloplasmin to maintain the vitality of tissues and organs. This review focuses on the structural and functional characteristics of the two proteins, with special emphasis on their coordinated regulation at the transcriptional and post-transcriptional level. Ceruloplasmin (CP) is a glycoprotein that plays an essential role in iron homeostasis. According to the accepted theory, the bivalent iron transported from the cell by ferritin, it is necessary to oxidize certainly by ceruloplasmin in order to slightly facilitate the activity of transferrin. Therefore, the ceruloplasmin-feroportin system is the main pathway of cellular iron metabolism and is responsible for regulating iron levels in the cell. Oxygen is a paradox for cells in that it is both an essential nutrient needed for survival and a precursor for toxic, potentially deadly byproducts. Ceruloplasmin represents a protein with specific domains capable of both facilitating the production of cellular energy and preventing the formation of oxygen radicals. This ability to perform dual tasks lies in the complex shape and structure of the protein and involves strategically placed copper ions, which can help both give and take up electrons from substrates, including iron, oxygen, and iron-binding proteins. Copper is the essential element for the wide range of ceruloplasmin activities that maximizes iron metabolism. A defect or mutation in the ceruloplasmin gene that denies copper incorporation into ceruloplasmin disrupts iron metabolism. Ceruloplasmin is also involved in many redox reactions. Its effect as a pro-oxidant or antioxidant is due to the presence of other factors. In the presence of superoxide (for example, in the inflamed vascular endothelium), it will act as an oxidation catalyst for low-density lipoproteins. About 95% of all copper in the body is found in connection with apoceruloplasmin, therefore, determining the amount of ceruloplasmin is one of the main methods of assessing copper exchange.

Key words*: ceruloplasmin, copper, iron, ferritin, oxidation.*

INTRODUCTION

The production of ceruloplasmin occurs in the liver. Hepatocytes largely synthesize ceruloplasmin. In the liver, P-type ATPase enzymes are required to incorporate copper into apoceruloplasmin (Maio et al., 2010). It is mainly synthesized in liver parenchymal cells, with small amounts coming from macrophages and lymphocytes (Vlasova et al., 2019). After the peptide chain is synthesized, copper is added from an intracellular ATPase (Das et al., 2018). Copper is essential for the normal folding of ceruloplasmin, as well as for the normal attachment of oligosaccharides. A large part of apoceruloplasmin, which does not contain copper or ATPase, undergoes intracellular degradation, although a small part will reach in the circulation, but has a short half-life of 4 to 5 days. Copper plays an important role in the body's redox processes due to its ability to change valence and thus to be a donor and acceptor of electrons (Harvey et al., 2008). In addition, copper is of great importance: for antioxidant protection (superoxide dismutase, ceruloplasmin, metallothionein), connective tissue formation (lysyl oxidase), electron transport (cytochrome C oxidase), blood coagulation, deamination of primary amines (amin oxidase) et al. (Филатов,

2010). Copper is also an integral part of enzymes, participating in the metabolism of vitamins, hormones, proteins, carbohydrates, as well as some immune processes. About 90% of the copper in the blood plasma is part of ceruloplasmin (Cu-alpha-2-globulin complex), the rest is free (Камышников, 2009). A change in the concentration of copper indicates possible pathological processes in the body, for the control of which it is necessary to screen the content of copper and/or ceruloplasmin.

Ceruloplasmin is then released into the bloodstream to be transported to the distal sites and to perform its functions in other metabolic processes, namely iron metabolism.

A form of ceruloplasmin anchored to GPI was also initially identified in Sertoli cells (Fortna et al., 1999).

Sertoli cells are the somatic component of the seminiferous tubule and are believed to provide physical and biochemical support to the process of spermatogenesis. Sertoli cells are closely associated with developing germ cells and are known to synthesize and secrete a number of proteins considered to be essential in maintaining and controlling spermatogenesis (Griswold, 1988).

Known proteins would be secreted by Sertoli cells in rat testes, which include transferrin, ceruloplasmin, sulfated glycoprotein-1 (SGP-1), sulfated glycoprotein-2 (SGP-2) and androgen binding protein (ABP). Transferrin and ceruloplasmin are transport proteins of metals, which bind iron and copper, respectively. SGP-1 has been identified as a precursor for the sulfatide/GMi activating protein, which is a necessary component in the degradation of glycosphingolipids (Collard et al., 1988). SGP-2 can be detected by immunofluorescence on mature sperm (Sylvester et al., 1984) and has recently been shown to have identity with SP-40,40, a protein that can inhibit complement-mediated cytolysis (Collard et al., 1987; Kirszbaum et al., 1989). ABP binds androgens and effectively increases the concentration of androgens in the testicular fluid (French et al., 1973). Changes in the levels of these proteins may be useful measures for the hormonal and environmental regulation of Sertoli cell function.

Transferrin is an important marker for the function of Sertoli cells, as its synthesis and secretion can directly affect germ cells. Transferrin was first demonstrated as a product of Sertoli cell secretion by Skinner and Griswold (Skinner et al., 1980). Tight intracellular junctions between adjacent Sertoli cells prevent access of serum transferrin in the adluminal compartment, thus testicular transferrin has the proposed function of delivering iron to germ cells. A model has been proposed (Huggenvik et al., 1985) in which iron, which was delivered to the basal part of the Sertoli cell via serum transferrin, is bound to testicular transferrin for subsequent delivery to the adluminal compartment. Many important elements of this model have been verified experimentally (1 and references in it). Iron delivery is essential for the process of spermatogenesis and therefore the means by which transferrin is regulated is of great interest. The adjustment of transferin synthesis in Sertoli cells has been studied by RIA hybridization and nucleic acid. Sentoli cells respond to FSH, insulin, retinol and testosterone with increased mRNA production of transferin protein both in vivo and in vitro (Huggenvik et al., 1985). In situ hybridization confirmed that transferrin is specifically produced by Sertoli cells in the testis and in addition this synthesis varies according to the stages of the seminiferous epithelium cycle. (Morales et al., 1987).

Regulators of Sertoli cell function may ultimately influence the maintenance and control of germ cell development. Germ cells (Galdieri et al., 1984; Ireland et al., 1987; Le Magueresse et al., 1988), including a number of factors such as vitamins (Hugly et al., 1987) and hormones (Fakunding et al., 1976), have been shown to regulate the function of Sertoli cells. Thus, it has been described that germ cells stimulate the phosphorylation of Sertoli cell-specific proteins in a Ca^{2+} dependent and cyclic adenosine monophosphate (cAMP) independent manner in culture studies (Ireland et al., 1987). Also, germ cells provoke an increase in the secretion of ABP with a concomitant decrease in the production of estradiol (Galdieri et al., 1984; Le Magueresse et al., 1988). It has also been shown that transferrin secretion is increased when Sertoli cells are cultured in the presence of germ cells or the conditioned germ cell environment

(Djakiew et al., 1988; Le Magueresse et al., 1988). It is now clear that germ cells can influence neighboring Sertoli cells to increase protein secretion. However, the mechanism by which germ cells mediate their effect has not been characterized.

Regulation of gene expression in mammalian testis is probably the result of complex interactions between several types of cells and their respective functional states.

In general, among the various functions of ceruloplasmin, the following main ones can currently be highlighted (Вавилова et al., 2005; Санина et al., 1986): 1) transport and regulation of copper turnover in the blood and organs; 2) ferrooxidase action and immobilization of serum iron; 3) antioxidant action; 4) participates in acute phase reactions; 5) increases the stability of all cell membranes. The properties of ceruloplasmin deserve great attention, on the basis of which it was classified as an acute phase protein of inflammation. It has been established that the level of ceruloplasmin in the blood serum changes significantly in different infectious diseases, processes accompanied by destructive and necrotic changes of tissues et al. (Вавилова et al., 2005).

Ceruloplasmin has a pronounced oxidase activity, in plasma it also limits the release of iron reserves, activates the oxidation of ascorbic acid, noradrenaline, serotonin and sulfhydryl compounds, also inactivates reactive oxygen species, preventing lipid peroxidation.

The insufficiency of copper ions in the blood, as a result of the deficit of ceruloplasmin, causes the increase of their absorption in the intestine, which favors even more their accumulation in the body with the subsequent influence on a series of important vital processes in the body (Камышников, 2009).

Ceruloplasmin (also called ferroxidase) catalyzes the oxidation of ferrous ions to a high level:

$$CP–Cu^{2+} + 4Fe^{2+} \rightarrow CP–Cu^{+} + 4Fe^{3+}$$
$$CP–Cu^{+} + O_2 + 4H^{+} \rightarrow CP–Cu^{2+} + 2H_2O$$

The metal ions with variable valence are able to effectively catalyze the prooxidant products, in particular, the hydroxyl radical ($^{\bullet}OH$). By promoting the incorporation of oxidized Fe^{3+} into ferritin, ceruloplasmin inhibits superoxide and ferritin-dependent lipid peroxidation. The properties of ceruloplasmin described above served as a basis for explaining its anti-inflammatory activity, which together with a rapid increase in its concentration (2-3 times) in the blood, already at the beginning of the inflammatory reaction, allows it to be classified as an "acute phase"protein.

Transferrin is also a glycoprotein in blood plasma, which binds firmly but reversibly to iron cations and provides the transport of these ions into the body of mammals. The polypeptide chain of the transferrin molecule consists of two Fe^{3+} binding sites. Transferrin is synthesized mainly in the liver. The level of expression of this Fe^{3+} binding protein is determined by the iron content in the body's biological media - it increases in iron deficiency states and decreases with an excess of this metal with variable valence. Saturated Fe^{3+} transferrin can penetrate through endocytosis only into those cells on the cytoplasmic membrane of which the specific transferrin receptor (TFR-1) is expressed.

Together with ceruloplasmin, it improves the binding of iron ions with transferrin, and in the case of their high concentration in the blood plasma with ferritin. It is believed that the blood plasma proteins ceruloplasmin and transferrin, together with tissue ferritin, form a ferrocinetic system, the main antioxidant system that controls the processes of lipid peroxidation induced by ferrous ions. Acting as a ferroxidase, ceruloplasmin plays an important role in the regulating of the ionic state of iron - the oxidation of Fe^{2+} to Fe^{3+}. This makes it possible for iron to be incorporated into transferrin without the formation of toxic iron products.

Chelate compounds, which have the ability to bind metal ions with variable valence (ferritin, hemosiderin, transferrin, ceruloplasmin, lactic and uric acid) are the most important component of the body's antioxidant system, as they neutralize the main catalysts of oxidation of free radicals in the body.

The iron released by transferrin is bound to a specific protein, ferritin, which supplies iron to the mitochondria, it is incorporated into the heme with the participation of ferrohelatase. The storage of iron in oxidized form prevents

its involvement in oxidative processes (Карбышев et al., 2018).
Under physiological conditions, transferrin is saturated with iron at a rate of about 30%. Transferrin carries out the exchange of iron, transporting it between the erythroid elements of the bone marrow and macrophages. Together with pathological changes, the transaction of iron from macrophages to transferrin is interrupted. This can occur during inflammation due to a decrease in the iron content in erythrocytes and an increase in its deposition in cells. Transferrin also regulates the transport of iron in hepatocytes.
The displacement of iron atoms in the cell occurs due to the interaction of the iron-transferrin complex with specific receptors on the plasma membrane. The iron-transferrin complex enters the cytosol of the cell, where an iron atom is released, and the transferrin is removed from the cell, remaining capable of repeated and multiple binding of iron ions. Reticulocytes have the highest density of transferrin receptors on the plasma membrane. The iron in these cells binds to protoporphyrin to form heme, which combines with globin to form hemoglobin or myoglobin.

MATERIALS AND METHODS

To carry out these researches in the experiments were used 10 roosters, which were divided into two groups of five animals in each group - control group and experimental group. To roosters from the experimental group were administered "per os" in a dose of 1 ml of green walnut hydroalcoholic extract with a total polyphenol content of 548.37 mg/100 g gallic acid equivalent (GAE). The extract was diluted in a ratio of 1/4 with distilled water and administered with the automatic device for the administration of medicinal products to animals.
The determination of ceruloplasmin was performed according to the process described by (Колб et al., 1982), the principle of the method is based on the fact that this component of blood plasma possessing oxidative properties, catalyzes the oxidation reaction of some polyamines, including p-phenylenediamine. As a result of the reaction are formed compounds of blue - violet color, the intensity of which correlates with the activity of the enzyme and is estimated spectrophotometrically.
The determination of antioxidant capacity (AOC) was performed by the CUPRAC (CUPric Reducing Antioxidant Capacity) method according to the process described by (Apak et al., 2005). The principle of the method is based on the property of antioxidants in the research sample to reduce bivalent copper to monovalent copper in the presence of the chelating agent - neocuproine. This chelator forms stable colored complexes with the monovalent copper ion which has a maximum absorption at 450-490 nm. The intensity of the staining is directly proportional to the AOC (Apak et al., 2005).

RESULTS AND DISCUSSIONS

In these researches the antioxidant activity of polyphenols from hydroalcoholic extract of green walnuts was evaluated according to several indices of the antioxidant system of breeding roosters. The research results are presented in Table 1.

Table 1. Indices of the antioxidant system in the blood serum of breeding roosters

Group	CP, mg/L	CUPRAC, mM/L	SOD, u/c (min/L)	Zinc, µM/L	Phosphorus mM/L	Iron, µM/L	Mg, mM/l	SH-thiol groups of proteins, µM/g
Control	108.9±0.35	1.3±0.32	153.1±0.52	32.73±0.88	1.65±0.53	12.63±0.68	0.733±0.0085	4.69±0.35
Experimental	123.53±1.54	2.71+0.20	171.3±0.92	35.39±0.80	1.82±0.12	15.55±0.88	0.896±0.048	5.78±0.28

Ceruloplasmin (CP) is a blood protein that contains copper and performs multiple fermentative functions in several important vital processes. Copper provides the fermentative function of ceruplasmin, which in turn distributes copper among the intercellular

spaces of the organism. One of the main functions of ceruloplasmin is the oxidation of iron to Fe^{3+}, after it is transported by transferrin to form hemoglobin. Transferrin is the main protein for iron, it can bind only with the oxidized form of Fe^{3+}. From the intestine iron comes out in the form of Fe^{2+}, in such a form it is not available to transport, therefore it is oxidized by ceruloplasmin and is included in the metabolism. In addition, ceruloplasmin performs the role of transport of copper, part of which distributes it for the synthesis of other enzymes (cytochrome-C-oxidases). In addition to iron, ceruloplasmin activates the oxidation of ascorbic acid, noradrenaline, serotonin and other compounds.

As a result of the research and the experimental material obtained, it is observed that polyphenols quantitatively influence the ceruloplasmin content, which in turn through fermentative reactions influences other indices of the antioxidant system. CUPRAC (CUPric Reducing Antioxidant Capacity) is increased in the experimental group up to 123.53±1.54 compared to the control group with a value of 108.9±0.35 mM/L. At the same time, the content of superoxide dismutase (SOD), zinc, phosphorus, iron, magnesium and SH-thiol groups of proteins is increasing.

Along with the indices of the antioxidant system, the hematological indices were also evaluated. The research results are presented in Table 2.

Table 2. Hematological indices of breeding roosters included in experiments to study the action of polyphenols from hydroalcoholic extract of green walnuts on the body

Hematological indices	Group	The obtained results				
Erythrocytes, 10^12/L	pre-experimental	3.22±0.33	3.45±0.25	3.62±0.12	3.58±0.04	3.28±0.02
	experimental	3.64±0.32	3.67±0.08	3.95±0.08	3.93±0.17	3.4±0.11
Hemoglobin, g/L	pre-experimental	138.6±3.51	139.3±5.5	142.0±3.0	145.3±3.05	136±3.0
	experimental	151±2.0	143.3±2.08	151.6±2.51	157.6±3.2	142.3±10.1
Hematocrit, %	pre-experimental	44.9±1.6	46.3±2.25	46.13±0.8	48.1±1.4	44.6±0.55
	experimental	49.5±1.73	47.2±1.22	49.8±0.63	51.6±2.9	46.0±2.0
Mean corpuscular volume MCV, fl	pre-experimental	132.4±0.69	131.5±0.87	129.6±0.38	134.1±2.55	135.8±0.40
	experimental	130.0±1.12	128.4±1.58	126.0±1.81	131.3±1.7	135.0±1.81
Mean corpuscular hemoglobin MCH, pg	pre-experimental	38.9±0.65	39.4±0.57	39.6±0.41	41.1±0.85	41.5±0.47
	experimental	39.6±0.16	39.0±0.21	38.4±0.17	40.1±0.62	41.7±0.75
Mean corpuscular hemoglobin concentration, g/dL	pre-experimental	30.7±0.35	30.5±0.16	30.7±0.45	30.5±0.35	30.6±0.38
	experimental	30.5±0.30	30.3±0.45	30.5±0.30	30.6±1.15	30.9±0.79
Erythrocyte distribution - standard deviation RDW-SD, fl	pre-experimental	42.8±0.38	41.3±0.51	43.5±0.36	45.0±3.63	43.1±0.55
	experimental	42.1±1.17	39.6±1.77	42.3±2.53	42.0±0.60	41.2±1.2
Erythrocyte distribution - coefficient of variation RDW-CV, %	pre-experimental	9.1±0.39	9.0±0.40	9.6±0.22	9.7±0.75	8.8±0.21
	experimental	8.9±0.23	8.5±0.45	9.2±0.36	8.7±0.07	8.5±0.36
Reticulocyte RET, 10^6/L	pre-experimental	0.8±0.09	0.9±0.12	1.1±0.18	1.09±0.21	0.9±0.13
	experimental	1.1±0.12	1.3±0.26	1.2±0.11	1.1±0.35	1.1±0.33
Immature reticulocyte fraction IRF, %	pre-experimental	4.5±0.21	5.8±0.31	4.9±0.28	6.1±0.45	5.4±0.27
	experimental	5.9±0.34	6.9±0.45	6.1±0.37	7.2±0.38	6.8±0.31

From the obtained results we observe that ceruloplasmin by activating the antioxidant fermentative system, has a pronounced influence on the hematological indices. These changes are observed depending on the group and for each individual animal. For example, the animal with the number one in the control group (pre-experimental) has an erythrocyte content of 3.22±0.33 10^12/L and corresponding to the animal with the number one in the experimental group, an increase in erythrocytes up to 3.64±0.32 10^12/L is observed, as well as at the other animals individual changes of erythriocyte concenration

are observed. Anological changes are also observed in the other hematological indices of the breeding roosters.

At the same time, the volume and mobility of the reproductive cells in the ejaculate of the breeding roosters were studied. The research results are presented in Table 3.

Table 3. Physiological indices of the spermogram of breeding roosters, which were given hydroalcoholic extract of green walnuts

Spermogram indices	Interval, days							
	norm	I	10	20	30	40	50	60
Volume, ml	0.5	0.5±0.07	0.8±0.08	0.8±0.07	1.2±0.12	1.3±0.16	1.3±0.15	1.2±0.12
Total mobility		26.1±0.32	47.0±0.30	50.0±0.24	58.3±0.26	71.4±0.42	89.8±0.26	91.2±0.26

Therefore, significant changes in the volume and total mobility of the reproductive material of breeding roosters are observed. All these changes are observed from the first day of administration of the preparation and during about two cycles of spermatogenesis. So, as you can see, there is an increase and an improvement in these indices.

CONCLUSIONS

Ceruloplasmin has a beneficial influence on the functioning and stabilization of the fermentative antioxidant system, which is manifested by detoxification of the animal organism at the cellular level.

Ceruloplasmin influences the hematological indices through its apparent influence on copper and iron metabolism.

The administration of hydroalcoholic extract of green walnuts, which contains polyphenols, beneficially influences the quality of the reproductive material.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 20.80009.7007.25 “Methods and procedures for maintenance and conservation of biodiversity depending on the integrity of gametogenesis and food variability”.

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