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THE INFLUENCE OF POLYPHENOLS OF NETTLE EXTRACT (*Urtica dioica*) ON THE ANTIOXIDANT ACTIVITY IN THE BLOOD SERUM OF ROOSTERS

Ion BALAN[1], Nicolae ROȘCA[2], Vladimir BUZAN[2], Ion MEREUȚA[2], Sergiu BALACCI[2], Roman CREȚU[2], Gheorghe BACU[2], Vlad TEMCIUC[2], Artiom FILIPPOV[2], Ecaterina VÎHRIST[2]

[1]Technical University of Moldova, 168, Stefan cel Mare Blvd, MD-2004, Chișinău, Republic of Moldova
[2]Moldova State University, Institute of Physiology and Sanocreatology, 1 Academiei Street, MD-2028, Chișinău, Republic of Moldova

Corresponding author email: vladimirbuzan@yahoo.com

Abstract

In conditions of intensification of growth and improvement of farm animals and birds, in order to maintain the immune status of the organism it is necessary to monitor the state of the body`s antioxidant system. The concentration of free radicals in the cells of the body can reach levels, at which the own antioxidant system is not able to deactivate the damaging agents, as a result of which oxidative stress can occur. Different remedies are used to correct oxidative stress, including natural and synthetic of various chemical nature, possessing antioxidant activity (AOA). The biochemical structure of vegetable origin remedies is close to the structure of metabolites of living organisms, which is conditioned by adaptation through evolution and correspondingly these remedies are more easily submit to the influence of fermentative systems, compared to synthetic analogues. In this way, the problem of researching and studying new opportunities and phytoprotective sources of natural antioxidants is currently being pursued. In this paper will be elucidated results about the influence of polyphenols extracted from nettle on the antioxidant activity in the blood serum of roosters.

Key words*: antioxidant activity, oxidative stress, polyphenols.*

INTRODUCTION

Oxygen free radicals (OFR) have important functions in maintaining the body's homeostasis and regulatory processes. It is certain the involvement of OFR in apoptosis, induction of genes responsible for immunological protection, participation in the degradation of phagocytosed compounds, recruitment of leukocytes to inflammation sites, activation of ion transport systems, renewal of biological membranes, activate as second messengers etc. (Jones, 2008). The intensification of peroxidic oxidation of lipids (LPO) in various pathological processes and diseases is accompanied by the accumulation in tissues and biological fluids of LPO products, such as lipid hydroperoxides, diene conjugates, carbonyl compounds and malonic dialdehyde. The significant increase of LPO products has been established in various pathologies, including, with disruption of the physiological mechanisms of living organisms (Cabre et al., 2000).

Currently, special attention is paid to the research of the imbalance in the lipid peroxidation (LPO) - antioxidant defense (AOD) system, as a factor of chronicization of the pathological process. The relevance of studying disorders of the LPO-AOD system is determined by their connection with inflammation and destruction of membranes, the negative impact of peroxidation products on various structures (inactivation of key enzymes of glycolysis), participation in the immune conflict, and also the possibility of developing other pathologies. Tissues and organ systems have different sensitivities to the effects of reactive oxygen species (ROS). This is probably due to the dynamic perception of the expression of antioxidant enzymes and the specificity of the metabolism of different tissues, which is ensured by the intracellular redox potential (redox potential), which is a

derivative of all the biochemical reactions of the cell.

The physiological state of the organism can be assessed by analyzing the imbalance between the LPO processes and the antioxidant system (Uglanova et al., 2010).

The state of lipid peroxidation is assessed by the content of lipid peroxidation intermediate products, which may include: lipid hydroperoxides, aldehydes, ketones and a number of low molecular weight acids (formic, acetic, butyric). These products are toxic agents that disrupt the functionality of membranes and the integrity of metabolism. In this case, the synthesis of conjugated diene compounds, peroxide radicals, malondialdehyde, etc. takes place. The synthesis of conjugated dienes reflects the initiation of oxidation steps.

Conjugated dienes, which are the primary products of lipid peroxidation, are toxic metabolites that have a detrimental effect on lipoproteins, proteins, enzymes and nucleic acids (Kudaeva & Masnavieva, 2015).

Free radicals cause oxidative damage to lipids, proteins and nucleic acids. Reactive oxygen species attack or damage all cellular components and, primarily, membrane lipids. Lipids (cholesterol, polyunsaturated fatty acids), the main component of cell membranes, located in close proximity to mitochondria, are the main target of oxidative attack with the formation and accumulation of lipid peroxidation products (LPO), especially hydroperoxides, oxysterols and endoperoxides (Barrera, 2012). Reactive oxygen species and LPO products alter the permeability and fluidity of the lipid membrane and significantly damage membrane function and cell integrity (Barrera, 2012).

Oxidative stress caused by increased levels of thyroid hormones is accompanied by aggravation and decreased quality of reproductive material, especially decreased in the mobility of male reproductive cells. A series of disturbances, which occur in living organisms and their systems, a series of medicinal remedies used in various pathologies of the animal organism, especially of reproductive males, can reduce the active potential of the reproductive system. With age, there are changes in the metabolism, which lead to a decrease in the amount of antioxidants, both enzymatic and non-enzymatic, which intensifies the increase in the level of oxidative stress, including in the testicles, which causes a decrease in the quality and quantity of male reproductive material (Bajenov & Filippova, 2018).

Oxidative stress primarily affects the membrane, which contains large amounts of polyunsaturated fatty acids, which leads to a decrease in sperm mobility. The inclusion of antioxidants in the food ration of breeding males or in the freezing-thawing mediums primarily influences the mobility and concentration of spermatozoa and increases the percentage of pregnant females with natural or artificial pregnancy (Ovcinnicok, 2022).

The inclusion of antioxidants in the food ration is recommended for breeding males but also in freezing-thawing mediums for a more efficient success of assisted reproduction. Reactive oxygen species (ROS) present in the ejaculate under natural conditions can cause oxidative stress when using assisted reproduction methods and technologies: an increase in ROS was recorded during cryopreservation and thawing of ejaculate and exposure to environmental factors and medium for freezing and thawing of reproductive material. The oxygen concentration in the medium for freezing and thawing reproductive material is much higher than that in the female reproductive tract (Bajenov & Filippova, 2018; Efremov et al., 2017).

Direct-acting antioxidants have immediate antiradical properties that can be detected in *in vitro* tests. Indirect antioxidants are understood as all compounds that reduce the development of oxidative stress *in vivo* (Shchulkin, 2018). A distinctive feature of this class of antioxidants is the ability to inhibit the processes of oxidation of free radicals, exclusively in biological objects (from cellular components to the whole organism).

The antioxidant protection of cells is a very effective system organized on several levels, and therefore the points of action of this system can be different. The most significant are direct endogenous antioxidants. Among them are α- and γ-tocopherols (vitamin E), which possess maximum activity. Retinols and β-carotene are of the second level as the most important endogenous fat-soluble antioxidants. The most

widely used in the practical field, oral antioxidant complexes have been obtained, which can be incorporated into the food ration (combined feeds), because they ensure the synergy of the components, due to the different mechanisms and levels of action, and at the same time ensure the greatest total antioxidant effect (Cekman et al., 2014; Shchulkin, 2018).

It should be noted that male reproductive cells have almost no antioxidant system of their own in the cytoplasm. But seminal plasma contains antioxidant defense components. Deficiency of these substances is associated with structural damage of reproductive material, such as membrane lipid peroxidation, protein denaturation and DNA fragmentation. Existing data indicate that ROS levels in seminal plasma are significantly higher in individuals with idiopathic infertility than in fertile breeders. The effectiveness of oral antioxidant complexes is due to increased protection against ROS, mainly in seminal plasma.

On the other hand, antioxidant enzymes (superoxide dismutase, catalase, peroxidase) have great activity and specificity, in relation to which they are expressed. Direct antioxidants have been suggested to have limited pharmacological capabilities. Indirect methods, such as normalization of mitochondrial function, are much more promising (Shchulkin, 2018). In this sense, the search for mitochondrial endogenous antioxidants is relevant.

Urtica dioica (stinging nettle) is a very widespread plant and present approximately all over the globe, whose benefits for maintaining the health of living organisms are of major importance. This plant, apart from the properties of maintaining the body's homeostasis through the ROS neutralization mechanisms, also possesses nutritional properties.

Chemical investigations revealed the presence of polyphenol carboxylic acids (caffeoylmalic acid, chlorogenic acid, ferulic acid and neochlorogenic acid) of flavonoids (rutin, isoquercitrin, astragalin), pelargonidin, epigallocatechin gallate, coumarins (scopoletin), sterols, carotenoids (β-Carotene, lycopene, lutein, neoxanthin, luteoxanthin) and lectins (Grevsen et al., 2008).

The presence of reactive oxygen species is related to various pathologies, including nutritional ones (metabolic pathologies). Free radicals act mainly by attacking the unsaturated fatty acids in the biological membrane which extend to the peroxidation of membrane lipids and finally to the cell inactivation or death. The mechanism of antioxidants is mainly the neutralization and elimination of free radicals (Kataki et al., 2012).

Nettle contains protein substances, amino acids, substances of carbohydrate nature, amines, sterols, ketones, volatile oil, fatty substances, formic, acetic, pantothenic and folic acid, vitamins A, B2, C, K, chlorophyll, mineral salts such as magnesium, calcium, iron, potassium, copper and silicon. The vesicant substance (for the skin) in the fresh plant consists of formic acid, an enzyme, and also a toxalbumin, responsible for the urticaria effect on the skin.

The nettle helps to detoxify the body, favors the transport of uric acid from the tissues into the blood circulation, thus increasing the elimination of uric acid from the body, especially of birds, which is related to the digestive and metabolic peculiarities of these animals from a physiological point of view, in order to prevent gout. It also stimulates the activity of metabolism, activates blood circulation, strengthens the immune system, and is used in the treatment of avitaminosis.

MATERIALS AND METHODS

The researches were carried out on 10 roosters, maintained *in vivarium* conditions in accordance with the prescriptions of the Regulation for the maintenance of laboratory animals. The roosters were distributed in cells of one individual each and were adapted to the maintenance conditions. The animals were divided into 2 groups: control group and experimental group. The animals from the experimental group were administered hydroalcoholic extract of polyphenols, obtained from nettle (*Urtica dioica*). The roosters in the experimental group were administered orally a dose of 1 ml per animal of hydroalcoholic extract from nettles, with a total polyphenol content of 33.2 mg gallic acid equivalent (GAE)/100 g. To exclude the excitation of the digestive tract, the extract was diluted with

distilled water in a ratio of 1:4. The administration was carried out with the automatic device for oral administration of medicinal remedies to animals.

The nettle was harvested from purely ecological areas and dried according to the requirements in force. For extraction, the dried nettle was ground with a coffee grinder. The separation of soluble polyphenolic compounds from plants can be achieved by diffusion of solid material (plant tissue) using a solvent that is selective for the target groups of compounds. Each plant material has certain specifics that can significantly influence the extraction of polyphenolic compounds (physico-chemical properties of target bioactive compounds, sensitivity to heat, light, enzymatic and non-enzymatic decomposition, oxygen concentration, etc.). Therefore, it is important to develop optimal extraction methods for the quantification and identification of polyphenols (Singleton et al., 1999). In order to obtain a hydroalcoholic extract with a high extraction efficiency of polyphenols, for experimental purposes, the ratio was constituted by 1:10 in hydroalcoholic solution with an alcohol concentration of 60%.

The determination of total phenolic compounds, expressed as gallic acid equivalent, was performed according to the Folin-Ciocalteu method. The Folin-Ciocalteu method was used to determine the total content of polyphenols in the extracts (Singleton et al., 1999). For this, 0.5 ml of the investigated solution was transferred into a 25 ml volumetric flask containing 10 ml of distilled water, where after that 0.5 ml of Folin-Ciocalteu reagent was added. After 5 min of rest, 8 ml of 7.5% sodium carbonate solution was added and thoroughly mixed. The volume of the flask was brought up to the mark with distilled water. After 2 hours, the absorbance was measured at the wavelength λ=765 nm. The total polyphenol content was estimated using the gallic acid calibration curve.

The total antioxidant activity (TAA) by the ABTS test is based on the ability of antioxidant compounds to annihilate the ABTS cationic radical and reduce the radical to the colorless neutral form. This test was performed according to Re's method (Re et al., 1999).

RESULTS AND DISCUSSIONS

In these researches, the antioxidant activity of polyphenols from the hydroalcoholic extract of nettle (*Urtica dioica* L.) was evaluated. The research results are presented in Table 1.

Table 1. Antioxidant activity of polyphenols from the hydroalcoholic extract of nettle (*Urtica dioica* L.) on biochemical indices in the blood serum of roosters

Group	Total protein g/L	TAA by ABTS, mM/L	TAA CUPRAC, mM/L	SOD, c.u. (min/L)	Catalase, μM/L
Control	54.64±0.68	396.31±0.56	1.01±0.16	55.62±0.61	11.72±0.53
Experimental	61.10±0.80	453.39±0.41	1.84±0.41	62.54±0.53	15.63±0.54

The role of proteins is very varied and consists in maintaining and ensuring the normal metabolism of the body but also in the maintenance of various biosynthetic pathways. They participate in the formation of immunity, provide intensive growth according to the species and breed. Proteins belonging to the Morpho-functional structures of living organisms, participate in biological processes, through the supply of amino acids that are used as plastic material for the growth of the locomotor system, the production of enzymes, the production of hormones, participate in immuno-reactive reactions, producing a series of antibodies, which fight against various infections of different nature. In the given research it is observed that the polyphenols extracted from the nettle influence the metabolism of proteins increasing them up to 61.10±0.80 g/L for the experimental group, where they can more certainly determine the capacity to meet the metabolic needs for amino acids and nitrogen, and for the control group constituting a value of 54.64±0.68 g/L, which

will influence the protein metabolic needs of the body by reducing them.

To evaluate the total antioxidant activity of phenolic compounds, different methods are used, which involve the use of various mechanisms for determining the antioxidant activity. In this case we used the $ABTS^{\bullet+}$ test (radical cation 2,2′-azinobis-(3-ethyl-6-sulfonate benzothiazoline) and the CUPRAC total antioxidant activity test, based on the ability to reduce the Cu ion by capturing the hydroxyl radical. As a result of our own research, we notice that the total antioxidant activity in the blood serum of roosters changes, obtaining an index of 453.39±0.41 mM/L in the experimental group, compared to the control group, which demonstrates a value of 396.31±0.56 mM/L, which proves to us that the polyphenols extracted from the nettle have a beneficial influence not only on the total antioxidant activity determined by the ABTS method, but also by the CUPRAC (Cupric Reducing Antioxidant Capacity) method, based on the capacity to reduce the Cu ion by capturing the hydroxyl radical, indicating an amount of 1.01±0.16 mM/L for the control group and a value of 1.84±0.41 for the experimental group.

As is known, oxygen is an indispensable element for all important vital processes in living organisms, especially for cellular and tissue respiration. However, oxygen metabolism can generate reactive elements called free radicals, especially the superoxide ion (O_2^-) and the hydroxyl ion (OH^-). These chemically unstable compounds carry free electrons that react with other molecules, destabilizing them in turn and thus inducing a chain reaction. In particular, free radicals damage DNA, essential cellular proteins and membrane lipids (lipid peroxidation), which can lead to cell death. Under physiological conditions there is a balance between the production of free radicals and endogenous mechanisms of antioxidant defense. These mechanisms mainly involve specific enzymes (superoxide dismutase, catalase and/or Gpx (glutathione peroxidase), as well as radical inhibitors such as vitamins with antioxidant properties, thiols, etc. The antioxidant system being also made up of hydrophilic antioxidant compounds, present in the cytoplasm of cells and blood serum, as well as hydrophobic compounds, which are localized in biological membranes, and strengthens its antioxidant capacities through the presence of superoxide dismutase (SOD) and catalase (CAT), being enzymatic antioxidants in the serum and cell cytoplasm, have a special contribution in the neutralization reactions of free radicals. As is known, when antioxidant defense systems are overloaded, oxidative stress occurs. This can ultimately contribute to the development of inflammatory diseases and other proliferative or degenerative biological dysfunctions. From the specialized literature it is proven that the superoxide ion (O_2^-) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by converting it to hydrogen peroxide (H_2O_2). The latter were then rapidly catabolized by catalase and peroxidases into Bio-Oxygen (O_2) and water (H_2O). Various studies have confirmed that the production under the action of SOD is the trigger of natural antioxidant defense mechanisms. Therefore, SOD is the enzyme in the natural defense against free radicals. The results obtained and presented in the table confirm a stimulating influence of superoxide dismutase activity, in the experimental group, compared to the control group, correspondingly indicating the following values, 62.54±0.53 and 55.62±0.61 c.u. (min/L). These enzymes act together, catalytically, which means that they are continuously regenerated.

Catalase is an important antioxidant enzyme responsible for the degradation of reactive oxygen species and hydrogen peroxide. It is present in all types of cells that contain the cytochrome system. Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen. Hydrogen peroxide (H_2O_2) being a by-product of various oxidase and superoxide dismutase reactions that occur inside the cell and is a normal product of cellular metabolism. The accumulation of H_2O_2 is extremely harmful to the cell and can lead to the oxidation of cellular proteins, lipids and DNA. This oxidative damage can eventually lead to DNA mutagenesis and cell death. To prevent this damage and survive, the cell relies on various antioxidants, including catalase. This enzyme has an important-vital function in cells, because

it catalyzes the decomposition of hydrogen peroxide into water and oxygen, always requiring the binding of two molecules of H_2O_2 at the active site to initiate this reaction. In the conducted research, a difference of catalase activity is observed in both groups, as follows for the control group a mathematical value of 11.72±0.53 μM/L was obtained, and for the experimental group it was a value of 15.63±0,54 μM/L, which demonstrates an obvious influence of polyphenols from nettle extract (*Urtica dioica*) on the antioxidant activity in the blood serum of roosters.

CONCLUSIONS

In this way, against the background of the appearance of various pathologies, the development of oxidative stress is observed, which is manifested by increasing the level of lipoperoxidation products and endogenous intoxication. In connection with this, it is necessary to evaluate in the complex the parameters of endotoxicosis and lipoperoxidation, including the level of conjugated dienes and trienes.

Apart from polyphenols and low molecular weight pigments, further research could also include other beneficial compounds present in nettle, such as oligomers and polymers, as well as sterols, for a complete insight into the bioactive and pharmaceutical potential of nettle.

The increased total antioxidant activity and the increase in the concentration of total proteins, which have an antioxidant role through different mechanisms, demonstrate the activation of the antioxidant system against oxidative stress.

The obtained results can be used to optimize the procedures for maintaining the body's homeostasis within the normal limits of the functioning of organs and organ systems in bio-objects.

SOD induces the activation of the endogenous antioxidant defense system. As a result, it is an enzyme that, fighting against overload with free radicals, and especially when the body's own natural protective forces are diminished under the influence of chronic stress, overwork, etc. By protecting and preventing chronic disorders, which involve oxidative stress, it stops their evolution, thus favoring the living conditions of bioobjects.

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REFERENCES

Bajenov, I.V., & Filippova, E.S. (2018). The role of oxidative stress in the pathogenesis of male infertility. *Effective pharmacotherapy. Urology and nephrology, 29*(3-4), 50-58.

Barrera, G. (2012). Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncology,* 1372-1389.

Cabre, M., Camps, J., Paternain, J.L., Ferré, N., & Joven, J. (2000). Time-course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. *Clinical and Experimental Pharmacology and Physiology, 27*(9), 694-699.

Cekman, I.S., Belenicev, I.F., Gorciakova, N.A., Kucerenko, L.I., Buhtiiarova, N.V., & Pogotova, G.A. (2014). Antioxidants: clinical and pharmacological aspect. *Ukrainian Medical Journal, 1*(99), 22-8.

Efremov, E.A., Kasatonova, E.V., & Melinik, I.I. (2017). Antioxidant therapy for male infertility as an opportunity to improve the outcomes of assisted reproductive technologies. *Effective pharmacotherapy. Urology and nephrology. Special issue "Men's health", 22*, 32-43.

Grevsen, K., Frette, X.C., & Christensen, L.P. (2008). Concentration and composition of flavonol glycosides and phenolic acids in aerial plants of stinging nettles (*Urtica dioica*) are affected by nitrogen fertilization and by harvest time. *European Journal of Horticultural Science, 73*(1), 20-27.

Jones, D.P. (2008). Radical-free biology of oxidative stress. *American Journal of Physiology-Cell Physiology, 295*(4), 849–868.

Kataki, M.S., Murugamani, V., Rajkumari, P.C., Mehra, P.S., Awasthi, D., & Yadav, R.S. (2012). Antioxidant, hepatoprotective and antihelmintic activities of methanol extract of *Urtica dioica* leaves. *Pharmaceutical Crops, 3*(1), 38 – 46.

Kudaeva, I.V., & Masnavieva, L.B. (2015). Methods for assessing oxidative status in laboratory practice. *Medical alphabet, 2*(1), 14–18.

Ovcinnicok, R.I. (2022). Male infertility associated with sperm oxidative stress: pathogenesis and therapeutic approach. *Medical Council, 5,* 46-53.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 10,* 1231-1237.

Shchulkin, A.V. (2018). A modern concept of antihypoxic and antioxidant effects of mexidol. *S.S. Korsakov Journal of Neurology and Psychiatry, 118*(12-2), 87-93.

Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152-178.

Uglanova, S.V., Popov, M.V., Kurova, S.V., Batrakova, E.V., Maniham, D., Kabanov, A.V., & Kliaciko, N.L. (2010). Stabilization of antioxidant enzymes in complexes and conjugates with block copolymers: prospects for the treatment of diseases of the central nervous system. *Moscow University Chemistry Bulletin, 51*(3), 227–234.