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INFLUENCE OF GREEN WALNUT EXTRACT ON THE ANTIOXIDANT STATUS OF THE ORGANISM OF BREEDING ROOSTERS

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Abstract

In recent times, the use of medicinal plants has significantly increased in correcting the disturbances in the organs and systems of living organisms, which can be caused by climate change, environmental pollution with various wastes, which contain heavy metals and other toxic substances released into the atmosphere as a result of processing of different raw materials et al. Researchers in this field have noticed that a number of plants have antioxidant, detoxifying properties, blocking properties of heavy metals from the internal circuit of living organisms and at the same time prevent the development of many disorders, which can cause different morbidity states of the body. In this paper will be analyzed and processed bibliographic scientific sources of researchers, which deal with the study of the physiological, biochemical, antioxidant, detoxifying, antiradical properties and the influence of biologically active components from Juglans regia L.. There will be presented some results obtained in the research conducted in our laboratory, on the influence of hydroalcoholic extract from green walnuts (Juglans regia L.), where will be taken into account the antioxidant influence and blocking properties of heavy metals, antiradical activity et al. The purpose of this paper was to determine the influence of biochemical complexes, which are contained in Juglans regia L. on antioxidant activity. From the results obtained after processing the specialized literature we notice that Juglans regia L. possesses high antioxidant properties, contain a series of biochemical compounds, such as vitamin C, flavonoids, quercetin et al. Due to the effect of Juglans regia L. on the amelioration of complications of various functional disorders in biological objects, it is necessary to further conduct complex physiological and biochemical studies on the use of Juglans regia L. components in various disorders in organs and systems of the human and animal organism.

Key words*: active forms of oxygen, antioxidants, antioxidant activity, extract, detoxification.*

INTRODUCTION

The walnut (*Juglans regia* L.) is a tree of the family Juglandaceae and contains many biologically active compounds with a particularly pronounced influence on the systems and organs of biological objects (Carvalho et al., 2010; Cosmulescu et al., 2010). Different parts of walnut are used as natural remedies in folk and classical medicine to balance some processes in living organisms possessing antidiarrheal, anthelmintic, antiseptic and astringent properties. Numerous studies have demonstrated the antioxidant potential of walnut products, i.e. hazelnuts (green or dried) and mesocarp of green walnuts (Ghasemi et al., 2011; Oliveira et al., 2008; Rahimipanah et al., 2008), leaves (Pereira et al., 2007), bark (Noumi et al., 2011) or flowers (Nabavi et al., 2011). According to studies conducted by (Rahimipanah et al., 2010), green walnut mesocarp is a valuable source of phenolic compounds and can be used as an alternative natural antioxidant in the pharmaceutical and food industries. Several studies suggest that regular consumption of nuts can have beneficial effects against disorders mediated by oxidative stress (Shimoda et al., 2009). Walnut fruits are impressively rich in polyphenolic compounds. It has been identified by Stampar et al. (2006) thirteen phenolic compounds in the green walnut mesocarp: chlorogens, caffeic, ferulic, synapic, gallic, ellagic, protocatechuic, syringic, vanylic acids, catechin, epicatechin, myricetin and juglone. The content of phenolic compounds depends on environmental conditions, genotype and stage of development of nuts (Solar et al., 2006), as well as geographical location or climatic conditions (Amaral et al., 2008). Regarding the seasonal variation in the content of phenolic compounds,

the highest content was determined in May and July (Amaral et al., 2008). Oliveira et al. (2008) have shown that the green walnut mesocarp is an important source for obtaining compounds with protective action for health, having high antimicrobial potential. It is proven, that walnuts have a higher total polyphenol content than most foods (Verardo et al., 2009).

Phenolic compounds have a major contribution to antioxidant activity because they are effective hydrogen donors (Banerjee et al., 2005). Phenolic acids, flavonoids and naphthoquinones are the main phenolic compounds of walnuts and walnut leaves. A special, unique component of walnuts is juglone (5-hydroxy-1,4-naptoquinone), a chemical compound released by walnut that can be toxic at various levels to many plant species. Juglone is present in considerable quantities in all green parts of the walnut (Cosmulescu et al., 2011) and in the walnut mesocarp, while the content of juglone in the endocarp is very low or absent (Jakopic et al., 2008). It is already proven (Colaric et al., 2005) that green walnuts are rich in phenolic compounds, and juglone is a well-known component of mesocarp, its content being significantly higher than the content of other phenolic compounds (Solar et al., 2006).

Juglone (5-hydroxyl-1,4-naphthoquinone), due to the antioxidant capabilities of phenolic compounds, juglone can serve to combat oxidative stress, thus protecting against the development of various diseases and aging processes. However, being a quinone molecule, juglone could also act as a redox cycle agent and produce reactive oxygen species. Juglon contains an intramolecular hydrogen bond between the hydroxyl and keto groups and is active in hydrogen atom donation (Jin et al., 2010). Juglon can have either pro- or antioxidant characteristics, depending on the concentrations (Chobot et al., 2009). Thus, some studies have reported the generation of ROS by juglone, while others describe its antioxidant properties (De Castro et al., 2004).

Juglone also activates mitogen-activated protein kinases that could promote cell survival, thus protecting against conditions such as heart damage.

By producing reactive oxygen species (ROS), biomolecules undergo oxidative stress. Antioxidants reduce ROS, and the balance between ROS and antioxidants defines oxidative stress. Phenolic compounds can inhibit these reactions by directly quenching ROS, inhibiting ROS-producing enzymes, chelating transition metal ions, transferring hydrogen atoms, and regenerating vitamin E (Tejero et al., 2007). Intramolecular hydrogen bonds play an important role in free radical stability (Nenadis et al., 2008).

Male reproductive cells are particularly sensitive to ROS-induced oxidation due to the presence in their plasma membrane of high levels of polyunsaturated fatty acids such as docosahexaenoic acid, which contains six double bonds per molecule (Aitken et al., 2014). Indeed, ROS mediates hydrogen by extracting from the hydrocarbon side chain a fatty acid, yielding to a carbon-centered lipid radical (L•) whose interaction with oxygen produces a lipid peroxyl radical (LOO•), capable of reacting with an adjacent fatty acid that propagates the process. As a result of internal molecular rearrangements, conjugated dienes and hydroperoxides are generated (Phaniendra et al., 2015). LPO products can also react with proteins, DNA and phospholipids, generating end products involved in cell dysfunction. The interaction of LPO products with amino residues can lead to protein oxidation, affecting the structural and functional characteristics of the protein (Niki et al., 2014). Similar 4-hydroxy-2-nonenal (4HNE) products are able to propagate ROS generation by interacting with proteins in the mitochondrial electron transport chain of spermatozoa (Aitken et al., 2014). Lipid peroxidation is strictly associated with changes in membrane fluidity and permeability, inhibition of membrane-bound enzymes and receptors, and activation of the apoptotic cascade, supporting the involvement of oxidative stress in spermatozoa mobility and morphology abnormalities (Nowicka-Bauer et al., 2020). Among LPO products, 4HNE appears to be highly responsible for cytotoxic effects on cells, sperm membrane, which induces loss of membrane integrity, changes in motility and compromises sperm-oocyte interactions (Baker et al., 2015; Walters et al., 2018; Nowicka-Bauer et al., 2020). It has been observed that the effects mediated by 4HNE

depend on several factors: the state of cellular differentiation, the amount of substrates for 4HNE attack and antioxidant defense systems (Walters et al., 2018).

The importance of oxidative stress in the etiology of disruption of male reproductive cell function was recognized because early studies by Thaddeus Mann and colleagues at the University of Cambridge demonstrated that mammalian spermatozoa are vulnerable to a process of lipid peroxidation that attacks unsaturated fatty acids in these cells, destroying the plasma membrane and compromising their functional competence. Induction of such stress may involve increased generation of reactive oxygen species (ROS) by these cells and/or a deficiency in the levels of antioxidant protection it provide. The net impact of oxidative stress includes a loss of motility, a decrease in sperm's ability to undergo acrosomal reaction, an impaired ability to fuse with the vitreous membrane of the oocyte, and DNA damage.

In addition to the negative influence, ROS also possesses regulatory functions of several intracellular processes, modifying the activation of various transcription factors (Burton et al., 2011) involved in intracellular signaling cascades for sperm physiology. In addition, ROS can improve the ability of sperm to bind to the pellucid area, inducing sperm-oocyte fusion (Wagner et al., 2018). By the way, antioxidant molecules can change the maturation of spermatozoa. In particular, catalase or superoxide dismutase (SOD) has been shown to inhibit sperm capacity or acrosomal reaction, supporting evidence of central involvement of ROS in sperm functioning (Wagner et al., 2018).

One of the major sources of superoxide anion in spermatozoa are mitochondria (Koppers et al., 2011). These organelles generate ROS as a normal byproduct of aerobic metabolism due to the leakage of electrons from the mitochondrial electron transport chain, which are then swept away by the universal electron acceptor, oxygen, to generate the superoxide anion. Mitochondrial ROS are also produced as part of the intrinsic apoptotic cascade that becomes activated whenever the phosphoinositide signaling pathway is compromised (Koppers et al., 2008).

Mitochondrial ROS generation and apoptosis may also be important in the mechanisms underlying sperm senescence. All mammalian spermatozoa have a finite lifespan and after a few days (depending on the species) will become senescent *in vivo* and *in vitro*, losing their viability, mobility, tyrosine phosphorylation state and DNA integrity with the passage of time (Matsuura et al., 2010; Yoshida et al., 2015). Oxidative stress appears to be a component of the senescence process, judging by the fact that sperm mobility and DNA integrity can be significantly improved in vitro if oxygen stresses are reduced and/or antioxidants are incorporated into the medium (Aitken et al., 2012).

MATERIALS AND METHODS

To achieve this purpose, hydroalcoholic extracts were obtained from green walnuts. The extract was administered to breeding roosters at a dose of one milliliter per head of the animal. To exclude irritation of the mucosa of the buco-esophageal tract the extract was diluted with distilled water. The experiments included two groups of five roosters in each - the experimental group and the control group.

Roosters from the experimental group were administered *per os* 1 ml of hydro-alcoholic extract from green walnuts, with a total polyphenol content of 548,37 mg/100g gallic acid equivalent (GAE). The extract was administered for two cycles of spermatogenesis, with the automatic device for the administration of drugs to animals. The total content of the polyphenolic compounds was determined by the Folin-Ciocalteu method. The method consists in determining the content of total polyphenols from plant sources by measuring the optical density of an extract which by complexation with Folin-Ciocalteu reagent absorbs in the VIS domain at wavelength $\lambda = 750$ nm. For the study was used the spectrophotometer "ПЭ-5400 УФ", with a spectral range from 190 to 1000 nm.

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).

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.

For the determination of biochemical indices were used biochemical analyzers "Sinergi" and "BioTECH", including sets corresponding to each index.

RESULTS AND DISCUSSIONS

The results of research of the fermentative antioxidant status in the blood serum of roosters given green walnut extract are presented in Table 1.

Tabel 1. Fermentative antioxidant status in the blood serum of roosters who received green walnut extract

Group	SOD, u/c (min/L)	G-GTP, u/L	Catalase, μM/L	G-S-T, nM/sL
Control	127.0±4.30	7.21±0.88	30.1±1.22	23.81±3.2
Experimental	156.0±3.71	10.4±1.09	35.1±1.1.1	34.2±4.18

As can be seen from the obtained data, the fermentative antioxidant status is very active in the control group. Superoxiddismutase is an enzyme that is part of the body's antioxidant defense system. It catalyzes the process of dismutation (disproportion) of the superoxide radical into hydrogen peroxide and molecular oxygen. Hydrogen peroxide is further converted into water and molecular oxygen under the action of catalase and glutathione peroxidase. The superoxide radical is produced in the process of oxidative energy reactions and is a product of the reduction of molecular oxygen by one electron. It is produced in almost all oxygen-consuming cells, can affect all cell components and the intercellular substance, and is also a precursor to the more toxic hydroxyl radical.

Tabel 2. Cationic and anionic antioxidant status in the blood serum of roosters

Group	Phosphorus, mM/L	Magnesium, mM/L	Zinc, μM/L	Iron, μM/L
Control	1.59±0.06	0.86±0.34	28.5±1.35	13.53±0.87
Experimental	1.69±0.06	0.87±0.5	34.7±1.03	15.28±0.77

It is known that about 80% of the total amount of phosphorus (700 g in the adult human) is deposited in the skeleton in the form of hydroxyapatite. The rest is found in extracellular fluid and soft tissues. Most phosphorus is combined with lipids, proteins and carbohydrates, participating in the formation of phospholipids, nucleotides and macroergic compounds. Phosphates are also one of the body's buffer systems.

Magnesium is involved in a wide range of biochemical reactions, having a direct activating impact on enzymes, such as phosphofructokinase, creatinine kinase and adenylate cyclase.

It intervenes in the synthesis, transport and use of macroergic compounds - ATP; ensures in the mitochondria the coupling of oxidation with phosphorylation.

It participates in the synthesis of nucleic acids and proteins, intervening in the activation of amino acids. Magnesium intervenes in ion transfer (K+) and modulates the activity of calcium channels. It has the mission of stabilizing cell membranes, as well as ribosomes and lysosomes.

Tabel 3. Protein antioxidant status, total and chemical antioxidant capacity in the blood serum of roosters in experiment no. 1

Group	CUPRAC,m M/L	CP, mg/L	AAT with ABTS, μM/L	SH-thiol groups of proteins, μM/g	Total protein, g/L
Control	1.3±0.32	108.9±0.35	116.1±0.71	4.69±0.35	53.4±0.51
Experimental	2.71+0.20	123.53±1.54	119.0±0.81	5.78±0.28	53.6±0.71

Ceruloplasmin being a protein of the blood, which depends on the copper content, performs its functions, contributing to the metabolism of other anions and cations, which promotes transmembrane metabolism and detoxification of cells and the organism as a whole. Copper, as usual, provides fermentative functions, including ceruloplasmin, which in turn distributes zinc through the intercellular spaces and also ensures the homeostatic status of the cell. It is necessary to note that ceruloplasmin oxidizes iron to Fe^{3+} after it is transported by transferrin to form hemoglobin. As a result of the research 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 compared to the control group, at the same time there are changes in AAT with ABTS, SH-thiol groups of proteins and in the total protein content.

CONCLUSIONS

Together with the evaluation of oxidative stress, the monitoring of redox status in the blood and ROS levels represent a new potential and a less invasive practice in the field of biomedicine to evaluate the functioning of the reproductive system and the quality of male reproductive material with the ability to fertilize.

Redox parameters can therefore be considered useful for the development of new strategies based on antioxidant supplementation to reduce systemic oxidative stress in males, improving the quality of reproductive material.

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