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# INFLUENCE OF SEA-BUCKTHORN FRUIT EXTRACT ON THE QUALITY OF MALE RABBIT SEMEN

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***Abstract***

*Obtaining high-quality reproductive material from different species of animals is one of the most important problems of purposefully solving and preserving the animal species, improving the quantitative and qualitative indicators of the resulting products of different animal species, and it can also be predetermined as an important factor in the conservation of biodiversity. This article presents an analysis of the results of the review of specialized literature and experimental data obtained in our laboratory. As a result of the impact of external negative factors on the functioning of the reproductive system of males and the quality of reproductive material, it encourages specialists and researchers in this area to conduct additional comprehensive studies of the reproductive potential of males and to prevent the occurrence of such disorders at any level of organization of the functioning of the reproductive system and the quality of spermatogenesis. All these disorders lead to a lack of antioxidants, which in turn lead to the formation and accumulation of reactive oxygen species (ROS).*

***Key words****: ecological disturbances, males, morphological changes, reproductive material, reproductive system.*

## INTRODUCTION

In order to maintain the constancy of the flow of processes to prevent the formation of ROS as a result of oxidative stress, any living organism needs to receive huge amounts of antioxidants from the outside. One of the richest in such substances are the sea-buckthorn fruits. It contains a lot of caratenoids, flavonoids and many other active substances.

Sea-buckthorn carotenoids include zeastaxanthin, lycopene, and carotenes, but are mainly represented mainly by β-carotene and exhibit antioxidant properties due to its content (Макаркина et al., 2011).

Sea-buckthorn berries contain a complex of flavonoid biologically active substances, such as catechins, leukoanthocyanidins, flavonols, flavonol glycosides. In particular, this complex is represented by such flavonoids as rutin, quercetin, hyperoside and astragalin (Корулькин et al., 2007). Flavonoids in sea-buckthorn fruits have anti-inflammatory, choleretic, antitumor, immunomodulatory and antimicrobial properties (Тринеева et al., 2012).

Flavonoids, in contrast to phenolic antioxidants (tocopherols), in addition to direct antiradical action, are able to bind metal ions with variable valence (transition metals), forming stable chelate complexes. It is known that the formation of such complexes of flavonoids with transition metal ions leads to the inhibition of free radical processes (Afanas'ev et al., 1989). Due to their chelating properties, flavonoids entering the body with food are able to affect the ion (metal) balance and the oxidative status of cells and tissues.

Metal complexes of flavonoids are significantly more effective interceptors of the oxygen anion radical than the initial complexones. In this case, the ligands in the complex are oxidized much more slowly than the free ligands. One of the features of the biological action of flavonoids is an extremely wide range of potential targets that they can act on in the body. On the one hand, this is due to the wide variety of plant pigments themselves, both in terms of their structure and redox properties. At the same time, each specific flavonoid is able to influence many structural and functional systems of the cell and the body as a whole. As an example, we can point to quercetin, one of the most widespread and studied flavonoids, which is contained in a rather impressive amount in sea-buckthorn fruits. Quercetin from numerous positive effects on different systems and organs of living organisms, through different mechanisms, has an anti-inflammatory effect, namely, it help to stabilize collagen production, inhibit platelet aggregation and stimulate the production of prostaglandins by the endothelium, which in turn lead to vasodilation (Rossi et al., 2003).

## MATERIALS AND METHODS

In the study used male rabbits of the New Zealand breed at the age of six months, which were injected with an aqueous extract of sea-buckthorn fruit. For obtain a water extract, we used frozen sea-buckthorn fruits at -50°C, collected from the central part of the Republic of Moldova, the harvest of 2019. The fruits were ground well in a porcelain mortar. The resulting mass was weighed and transferred to a conical flask, where distilled water was added at the rate of 1 g of the resulting mass and 5 ml of distilled water. It mixed well until a homogeneous suspension was obtained and kept in a water bath at 60°C for 40 minutes. The resulting extract was passed through five layers of gauze (for filtration) and, using a disposable syringe, 15 ml of the obtained extract was injected into male rabbits through the oral cavity for 60 days.

## RESULTS AND DISCUSSIONS

To date, special attention is paid to the mechanisms of ROS formation and their impact on the reproductive system and the process of spermatogenesis. A number of authors (Tremellen et al., 2008) have proved that as a result of any stress, endogenous initiators of inflammation are first released into the intercellular environment. This is followed by stimulation of phagocytosis, activation of neutrophil NADPH oxidase and, ultimately, the formation of reactive oxygen species - ozone, free radicals, hydrogen peroxide, etc. The imbalance between the production of free radicals and the weakening of antioxidant protection in various parts of the male reproductive system, regardless of the etiological factor, is the main indicator of oxidative stress, which has a positive correlation with the degree of infertility of males of different animal species. Excessive production of ROS, which causes damage of membranes, can lead to a decrease in the motility and fertilizing ability of spermatozoa (Tremellen et al., 2008). ROS can have a direct damaging effect on the DNA of chromosomes, and in addition, they are able to initiate endonuclease-mediated sperm apoptosis, which in the vast majority of cases can cause infertility. ROS are universal limiters of sperm count and regulators of ejaculate quality from the point of view of evolution (Tremellen et al., 2008). Since the main substrate for free radical oxidation are phospholipids, the intensity of lipid peroxidation processes will directly depend on their composition and structural organization, the violation of which can lead to a decrease of motility and quality of spermatozoa and, as a consequence, to infertility (Хышиктуев et al., 2010). Assessment of the level of oxygen free radical generation in the ejaculate is one of the important methods that allow us to characterize the fertility of sperm in the conditions of normospermia and pathospermia (Aitken et al., 2012).

The internal component of the antioxidant system is also very important. Glutathione peroxidase has a unique position in the mammalian reproductive system, as it is directly related to the acquisition and maintenance of sperm integrity. Unlike superoxide dismutase, which is rather a pro-oxidant, forming aggressive and stable $H_2O_2$ from short-lived superoxide, and the catalase, which is active only at high substrate concentrations, glutathione peroxidase destroys, in addition to hydrogen peroxide, other organic peroxides, even with a slight increase in their concentration, maintaining cell homeostasis (Miao et al., 2009; Sharma et al., 2006; Колесникова et al., 2013). Glutathione peroxidase forms the first response to oxidative stress and acts as a scavenger during ROS leakage and the development of chain uncontrolled processes. Glutathione-S-transferase is the most important polyfunctional protein of ejaculate, since it not only protects against xenobiotics and ROS, but also, being localized on the surface of spermatozoa, plays the role of a trigger that triggers their interaction with the ligands of the zona pellucida at the stage of initiation of the acrosomal reaction. That is why the determination of the content of glutathione-S-transferase can be used not only to check the antioxidant activity of drugs, but also to establish the fertilizing ability of spermatozoa (Wu et al., 2008).

The production of excessive amounts or accumulation of active substances that interfere with redox reactions can adversely affect macromolecules, cell membranes, and DNA (Valko et al., 2008).

All of the above can change the biological properties of membranes, enzymes and receptors, disrupt the functioning of the cell and lead to its death (Dalle-Donne et al., 2006).

The identification of free radicals as promoters of inhibition of the processes of cell activity and metabolic disorders in it, allowed us to come to the idea that their inactivation or complete blockade (correction of excessive oxidation reactions, or oxidative stress) can be a pathogenetic basis for effective prevention of the manifestation of most dysfunctions in the body of animals, including the state of the reproductive system and the quality of reproductive material. Redox reactions occur with a change in the degrees of oxidation of the atoms that are part of the reacting substances. The change is realized through the redistribution of electrons between the oxidizing atom (acceptor) and the reducing atom (donor), which are an integral attribute of the normal biochemistry of any healthy cell. Since living organisms are an aerobic system, it is oxygen that acts as a key oxidant in the course of cellular redox reactions. About 95% of all consumed oxygen in the cell is reduced in mitochondria to water during respiration and energy synthesis by the cell (in the form of adenosine triphosphate). The remaining 5% as a result of various redox reactions inevitably transform into reactive oxygen and nitrogen forms, which are designated by the general term "free radicals" (Bartz et al., 2010). Thus, the theory of oxidative stress, substantiating the key role of oxidative stress in the pathogenesis of most dysfunctions and necessitating the inclusion in the diet of plant-based foods rich in antioxidants. Increased oxidative stress leads to an imbalance between the production of free radicals and the antioxidant defense of the body (Sastre et al., 2000). At the same time, oxidative stress affects almost all structures of the body, including DNA, proteins and lipids (Weinert et al., 2003). There is evidence that oxidative stress can be an important factor leading to the development of new somatic mutations, which made it possible in 1998 to formulate another daughter theory of aging - the theory of somatic mutations. Thus, normally, the vast majority of biochemical processes in cells occurring under aerobic conditions are associated with the natural formation of free radicals in the course of physiological redox reactions (Rahal et al., 2014). However, some of the free radicals (primary free radicals) are constantly formed in the process of vital activity of the organism and are "useful" for it, since they participate in the whole spectrum of physiological reactions necessary for normal life: a) regulation of cellular processes (cell division, respiration) through signaling dependent on reactive oxygen species; b) providing a bactericidal and oncostatic effect; c) activation of immune reactions of leukocytes; d) providing an anti-inflammatory systemic and local response, etc. Among the most important "physiological" primary free radicals for the body are superoxide anion radical ($O_2$–), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), hypochloric acid (HOCl), nitric oxide (NO), peroxynitrite (ONOO-). Secondary free radicals, unlike primary ones, do not perform physiologically useful functions. On the contrary, they have a destructive effect on cellular structures, seeking to take away electrons from "full-fledged" molecules, as a result of which the "affected" molecule itself becomes a weak (tertiary) free radical. To protect against free radicals (endogenous) formed in the process of vital activity and free radicals coming from outside (exogenous), cells have a special system that inactivates the synthesis and negative effects of free radicals – an antioxidant system. This system is formed by low molecular weight antioxidants and specialized antioxidant enzymes. Key antioxidant enzymes include enzymes of a specialized enzyme system, which includes superoxide dismutase, catalase, and glutathione peroxidase, which are found in large quantities in the mitochondria. These enzymes are the first to begin to catalyze the biochemical reactions of free radical inactivation, as a result of which free radicals and peroxides are converted into inactive compounds (Меньщикова et al., 2006). In addition, there are numerous non-specialized cellular enzymatic systems for inactivation of free radicals, represented by low molecular weight antioxidants - vitamins A, E, K, C, D, steroid hormones, flavonoids, polyphenols (vitamin P, coenzyme Q10, or ubiquinone), thiol-disulfide system on based on glutathione (in particular alpha-lipoic or thioctic acid - ALA), aromatic compounds, uric acid, taurine, carnosine, acetyl-L-carnitine, L-acetylcysteine, natural chelators of iron, zinc, selenium, manganese, chromium ions and others (Jones, 2008; Костюк et al., 2004). Normally, in a living organism, the principle of the "golden triangle of oxidative balance" is always observed, according to which only a dynamic balance between the level of free radical production, the activity of the antioxidant defense system and the normal functioning of transmitter (transmitting biological signals) biomolecules can ensure the biological safety of the cell and the whole organism as a whole. In case of violation of the "golden triangle of oxidative balance" (hyperproduction and/or excessive intake of free radicals in the body in combination with an insufficient rate of their inactivetion due to a deficiency or depletion of the protective mechanisms of the antioxidant defense system, or a combination of all the above pathological processes), the physiological behavior of the course of redox reactions characteristic of a healthy cell disappears. This is accompanied by a loss of control over the metabolism of free radicals, which leads to a cascading and uncontrolled increase in them in the cell and the body, which is called "pathological oxidative stress" (Carmeli et al., 2002). Excessive oxidative stress affects almost all body structures, including DNA, proteins and membrane lipids. At the cellular and tissue level, it is manifested by various disorders of homeostasis:

1) an imbalance between anti-inflammatory cytokines;
2) endothelial dysfunction;
3) membranopathies due to activation of lipid peroxidation of cell membranes (hypoxia);
4) violation of cellular reception and perception;
5) disorders of the metabolism of biogenic amines;
6) energy and metabolic disorders;
7) disorders of telomerase activity of cell chromosomes.

For effective management of pathological cellular reactions underlying oxidative stress, it is proposed to use biological active substances of plant origin - anthocyanins, polyphenols, flavonoids etc., combined under the general name "antioxidants". Antioxidants can be numerous chemicals, including natural products of the body's activity, and nutrients from food, which neutralize the oxidative effect of free radicals and other harmful substances (Тюзиков et al., 2018). Antioxidants are divided into two large subclasses, depending on whether they are soluble in water (hydrophilic) or lipids (lipophilic). Water-soluble antioxidants are oxidized in the cell cytosol and blood plasma, while fat-soluble antioxidants protect cell membranes from lipid peroxidation on the surface (Костюк et al., 2004). Various antioxidants are present in a wide range of concentrations in body fluids and tissues, while some (glutathione or ubiquinone) are mainly localized within cells, while others (uric acid) are more evenly distributed (Меньщикова et al., 2006; Костюк et al., 2004). Antioxidants are a specific group of chemicals of various chemical structures that have a common property - the ability to bind free radicals and slow down pathologically excessive redox reactions. However, based on the results of well-designed experimental biomedical studies there is still insufficient evidence of the effectiveness of antioxidants in these processes. The choice of specific drugs and their use are not yet fully developed and require further experimental studies.

The scientific staff of our laboratory conducted experiments to study the effect of biological active substances obtained from sea-buckthorn on the reproductive system and the quality of reproductive material.

The data obtained are presented in Table 1.

Table 1. Functional parameters of sperm of male breeder rabbits that were given sea-buckthorn extract

| Sperm indicators | Interval, days | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | 10 | 20 | 30 | 40 | 50 | 60 |
| Volum, ml | 0.42±0.097 | 0.72±0.096 | 0.82±0.097 | 0.92±0.52 | 1.0±0.141 | 1.12±0.21 | 1.14±0.19 |
| Mobility, % | 49.66±1.196 | 50.92±1.93 | 55.44±0.88 | 76.36±0.74 | 82.6±0.56 | 88.86±0.79 | 94.08±0.58 |
| Spermatozoa speed, μm/s | 18.8±0.83 | 20.2±0.83 | 22.6±0.54 | 22.4±0.54 | 23.4±0.59 | 22.0±0.70 | 22.8±0.44 |
| Spermatozoa concentration, million/ml | 121.8±0.83 | 180.6±1.14 | 221.2±1.71 | 285.78±0.148 | 286.38±0.311 | 300.28±0.589 | 340.3±0.524 |

From the data of the table 1, it can be seen that the studied indicators of spermogram change significantly with a pronounced trend throughout the entire period of the study. The volume of the ejaculate increases on average from 0.42 ± 0.097 to 1.14 ± 0.197 ml, the motility from 49.66 ± 1.196 to 94.08 ± 0.58%, and the concentration of reproductive cells from 121.8 ± 0.83 to 340.3 ± 0.524 million/ml on the 60th day. The speed of movement of reproductive cells is maintained in the range of 18.8 ± 0.83 - 22.8 ± 0.44 μm/sec.

All these changes in the functioning and maintenance of the male reproductive system are caused by polyphenolic compounds that are contained in the composition of sea-buckthorn fruits, due to their antioxidant effect and possessing the main protective properties of the cell membrane, maintaining the stable functioning of cellular components due to the activation and deactivation of many enzymes of the antioxidant system, these substances support the homeostasis of the body as a whole.

## CONCLUSIONS

The body's antioxidant defense system are an important role in protecting cells from excess ROS and consists of an endogenous component (uric acid, superoxide dismutase, catalase, glutathione peroxidase, etc.) and an exogenous component (bioflavonoids, carotenoids, tocopherols, ascorbate, etc.).

Changes caused by free radicals affect lipids in the structure of cell membranes, cell organelles (mitochondria, lysosomes) and components of blood vessel walls.

The obtained data indicate a sufficiently high content of antioxidants of the flavonoid structure and indicate the prospects of using extracts from sea-buckthorn fruits to regulate and stabilize the functional activity of the reproductive system, the process of spermatogenesis in male rabbits and further to preserve biodiversity.

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## REFERENCES

Afanasev, I.B., Dorozhko, A.I., Brodskii, A.V., Kostyuk, V.A., & Potapovitch, A.I. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.,* 38, 1763–1769.

Aitken, R.J., Whiting, S., De Iuliis, G.N., McClymont, S., Mitchell, L.A., & Baker, M.A. (2012). Electrophilic aldehydes generated by sperm metabolism activate mitochondrial reactive oxygen species generation and apoptosis by targeting succinate dehydrogenase. *J. Biol. Chem.,* 39(287), 33048–33060.

Bartz, R.R., & Piantadosi, C.A. (2010). Clinical review: oxygen as a signaling molecule. *Crit. Care.*, 14(5), 234.

Carmeli, E., Coleman, R., & Reznick, A.Z. (2002). The biochemistry of aging muscle. *Exp. Gerontol.*, 37(4), 477–489.

Dalle-Donne, I, Rossi, R, Colombo, R, Giustarini, D, & Milzani, A. (2006). Biomarkers of xidative damage in human disease. *Clin. Chem.*, 52, 601–623.

Jones, D.P. (2008). Radical-free biology of oxidative stress. *Am. J. Physiol. Cell Physiol.*, 295 (4), 849–868.

Miao, L., & Clair, D.K. (2009). Regulation of superoxide dismutase genes: Implications in disease. *Free Radic. Biol. Med.*, 47, 344–350.

Rahal, A., Kumar, A., & Singh, V. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *Biomed. Res. Int.,* 1-19.

Rossi, A., Serraino, I., Dugo, P., Di Paola, R., Mondello, L., Genovese, T., Dugo, G., Sautebin, L., Caputi, A., & Cuzzocrea, S. (2003). Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. *Free Radical Research*, 37, 891–900.

Sastre, J., Pallardó, F.V., Garcia de la Asuncion, J., & Viña, J. (2000). Mitochondria, oxidative stress and aging. *Free Radic. Res.*, 32 (3), 189–198.

Sharma, R., Yang, Y., Sharma, A., Awasthi, S., & Awasthi, Y.C. (2006). Antioxidant role of glutathione-S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. *Antioxid. Redox. Signal*, 6, 289–300.

Tremellen, K. (2008). Oxidative stress and of male infertility. *Hum. Reproduct. Update,* 14 (3), 243–258.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., & Telser, J. (2008). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, 39, 44–84.

Weinert, B.T., & Timiras, P.S. (2003). Invited review: theories of aging. *J. Appl. Physiol.*, 95 (4), 1706–1716.

Wu, J.P., Xing–Tang, K.F., Xue, W., Liu, M., Sun, J.H., Wang, X.Y., & Jin, X.J. (2008). Genetic polymorphism of glutathione S-transferase T1 gene and susceptibility to idiopatic azoospermia or oligozoospermia in north western. *China Asian J. Androl.*, 10, 266–270.

Колесникова, Л.И., Баирова, Т.А., & Первушина, О.А. (2013). Этногенетические маркеры антиоксидантной системы. *Бюллетень ВСНЦ СО РАМН*, 4 (92), 166–171.

Корулькин, Д.Ю., Абилов, Ж.А., Музычкина, Р.А., & Толстиков, Г.А. (2007). *Природные флавоноиды.* Новосибирск: Академическое изд-во «Тео».

Костюк, В.А., & Потапович, А.И. (2004). *Биорадикалы и биоантиоксиданты.* Минск: БГУ.

Макаркина, М.А., Богомолова, Н.И., & Соколова, С.Е. (2011). Содержание витаминов С и каротиноидов в плодах различных сортов облепихи в условиях Средней полосы России. *Современное садоводство,* 1, 1–5.

Меньщикова, Е.Б., Ланкин, В.З., & Зенков, Н.К. (2006). *Окислительный стресс. Прооксиданты и антиоксиданты.* М.: Фирма «Слово».

Тринеева, О.В., Сафонова, И.И. et al. (2012). Определение флавоноидов в плодах облепихи крушиновидной. *Фармацевтическая химия и фармакогнозия,* 7, 18–21.

Тюзиков, И.А., Калинченко, С.Ю., Ворслов, Л.О., & Тишова, Ю.А. (2018). Роль окислительного стресса в патогенезе андрологических заболеваний. Тиоктовая (альфа-липоевая) кислота – новые грани фармакотерапевтических опций в современной андрологической практике. *Урология и Нефрология*", 9 (2), 20–37.

Хышиктуев, Б.С., & Кошмелев, А.А. (2010). Особенности изменений фосфолипидного состава семенной жидкости у мужчин с нарушением фертильности. *Клиническая лабораторная диагностика*, 7, 27–30.