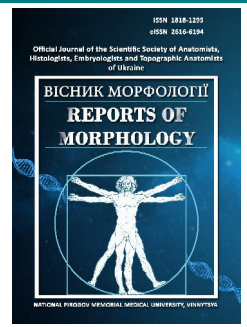




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The effect of quercetin on the morphogenesis of the interstitial space in the testes of rats after 90 days with central blockade of luteinizing hormone

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CONFLICT OF INTEREST

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Not applicable.

Leydig cells are responsible for the production of testosterone in the male testicles when stimulated by luteinizing hormone (LH). Dysfunction of Leydig cells, which occurs during inflammation or oxidative stress, is one of the main causes of male infertility. The purpose of the study is to determine the effect of quercetin on the microscopic organization of rat testes, nitric oxide production and the intensity of oxidative stress in rat testes on the 90th day of the experiment, during the experimental central deprivation of LH synthesis caused by the administration of triptorelin acetate solution. The experiment was conducted on 20 sexually mature male white rats. Rats were divided into 2 groups of 10 animals in each group: control group (I), group with central deprivation of LH + quercetin synthesis (II). Animals from the group with central blockade of LH synthesis were injected subcutaneously with triptorelin acetate at a dose of 0.3 mg of the active substance per kg and quercetin at 100 mg per kg of body weight 3 times a week, while the control group was injected with saline. Our study of the interstitial space in the testes of white rats showed heterogeneity of macrophage populations and variability of structural and functional parameters. Central blockade of LH synthesis by the administration of triptorelin with the parallel administration of quercetin to the studied animals on the 90th day of the experiment causes changes in the structure of the interstitial space of rat testes, which is characterized by high variability both in the populations of interstitial endocrinocytes and macrophages. Biochemical indicators on the 90th day of the experiment indicate an increase in NO production in conditions of central blocking of the synthesis of luteinizing hormone by more than three times, which is ensured by the activity of the inducible isoform of NOS (iNOS). At the same time, the increase in the activity of iNOS with a decrease in the activity of the arginase pathway leads to the polarization of macrophages according to the pro-inflammatory type. The introduction of quercetin protects the testicular tissue of rats from oxidative damage caused by the administration of triptorelin on the 90th day of the experiment by increasing antioxidant protection and reducing reactive oxygen species in the tissue. **Keywords:** testes, interstitial endocrinocytes, macrophages, quercetin, triptorelin, oxidative stress, rats.

Introduction

Male infertility accounts for 50 % of all infertility cases. The main reason is low quality and quantity of sperm. Leydig cells are responsible for the production of testosterone in male testes when stimulated by luteinizing hormone (LH) [2, 16]. Leydig cell dysfunction associated with inflammation and oxidative stress is one of the main causes of male infertility. Men with complete congenital hypogonadotropic hypogonadism usually have azoospermia. Treatment with only exogenous injection of testosterone and follicle-stimulating hormone (FSH) fails

to produce sperm. However, the combined treatment of FSH and human chorionic gonadotropin (hCG) is effective [3, 11]. This example shows that intratesticular testosterone plays an important role in spermatogenesis. In addition, testicular histology of men with LH receptor mutations shows hypoplasia, agenesis, dysplasia of Leydig cells with a preserved number of Sertoli cells; the number of sperm is reduced, as in men with a partial inactivating mutation of the androgen receptor.

Certain male hormonal contraceptive protocols or

exogenous androgen abuse cause a negative feedback loop in the hypothalamic-pituitary axis, reducing FSH, LH, and testosterone levels, causing sperm defects and testicular atrophy, leading to abnormal androgen production and impaired spermatogenesis. The minimum level of intratesticular testosterone for normal sperm production is a matter of debate. Interestingly, some animal models have shown that completely testosterone-independent spermatogenesis is possible, potentially due to strong FSH activation. Recent data indicate an important role of macrophages in adult spermatogenesis. Testicular macrophages regulate immune function during inflammation and steroidogenesis of Leydig cells [13, 17, 21]. Thus, inhibition of induced cytoglobin (CYGB) and neuroglobin (NGB), release of nitric oxide (NO) blocked the progression of the cell cycle, reduced testosterone production, and enhanced the expression of inflammatory and apoptotic pathway genes. On the other hand, overexpression of CYGB and NGB decreased the protein expression of the inflammatory cytokine tumor necrosis factor (TNF α) and the inducible enzyme COX-2 and increased the expression of testosterone biogenesis pathway genes under lipopolysaccharide stimulation. In addition, overexpression of CYGB and NGB enhances testosterone production. Inhibition of CYGB and NGB in Leydig cells changed the morphology of macrophages, increased the number of macrophage cells and NO release in lipopolysaccharide co-culture experiments.

One of the strategies developed to attenuate doxorubicin (DOX) toxicity is combination therapy with bioactive compounds such as flavonoids. The protective properties of some flavonoids against DOX toxicity have been investigated and observed mainly in the heart, but also in the liver, kidney, brain, testes or bone marrow [9, 22]. Protective mechanisms include reducing oxidative stress by reducing ROS levels and/or increasing antioxidant defenses and inhibiting autophagy, apoptosis, and inflammation [18, 23]. Cancer cell studies showed that the antitumor activity of DOX was not affected by flavonoids. Moreover, some of them enhanced the efficacy of DOX as an antitumor drug even in multidrug-resistant cells [26].

Quercetin has several pharmacologic actions that may help combat cell-related insults such as altered sperm function, reproductive hormone dysfunction, as well as dysregulated testicular apoptosis, oxidative stress, and inflammation. Studies have shown that quercetin reduces testicular toxicity, mainly by inhibiting the formation of reactive oxygen species with the help of two antioxidant pharmacophores present in its ring structure [12, 21]. Quercetin's free radical scavenging property can alter the signal transduction of oxidative stress-induced apoptosis, prevent inflammation, and improve sperm quality in a hormone-dependent manner. The protective effect of the bioflavonoid quercetin revealed on the experimental model makes it possible to use it to correct testicular dysfunction of various genesis [25]. These facts indicate the need to

take measures to identify the causes of reproductive abnormalities, study the mechanisms and find preventive means of correction.

The purpose of the study was to establish the influence of quercetin on the microscopic organization of rat testes, production of nitric oxide and the intensity of oxidative stress in the rat testes on the 90th day of the experiment, during experimental central deprivation of LH synthesis, caused by the introduction of triptorelin acetate solution.

Materials and methods

The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants action diphereline, ethanol and 1 % methacrylic acid on the morphofunctional status in a number of internal organs", state registration No. 0119U102925.

The experiments were carried out on 15 sexually mature male white rats. Rats were divided into 2 groups with 10 animals in each group: the control group (I), the group with central deprivation of LH synthesis + quercetin (II). Animals from the group with central deprivation of testosterone synthesis were injected subcutaneously with triptorelin acetate at a dose of 0.3 mg of the active substance per kg [5, 19] and quercetin 100 mg per kg body weight 3 times a week [20], while the control group was administered saline. Experiment conducted for 90 days. Animals were kept in standard vivarium conditions of the Poltava State Medical University.

Experimental animals were sacrificed in strict compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes"; (Strasbourg, 1986), as well as with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001). Bioethics protocol № 195 - 06.24.2021.

Using standard methods, the material was imbedded in paraffin blocks, of which sections 4 μ m thick were made and stained with hematoxylin and eosin [4]. Histological preparations were examined using Biorex 3 light microscope with digital microfilter with software adapted for these studies (Serial No. 5604).

We carried out all biochemical studies in 10 % homogenate of testis tissue using ULab 101 spectrophotometer. General activity of NO-synthase (gNOS), activity of constitutive isoforms (cNOS), activity of inducible isoform (iNOS) was determined by increase of nitrite concentration after incubation in buffer solution (pH=7.4) containing 0.3 ml of 320 mM L-arginine solution and 0.1 ml of 1 mM NADPH+H solution [1, 24]. Nitrite concentration was measured with help of Griess reagent [1, 24]. Arginase activity was evaluated by increase of L-ornithine content after incubation in buffer solution (pH=7.0) containing 0.2 ml of 24 mM L-arginine solution [1, 24].

Basic production of superoxide anion radical (SAR), its production by the mitochondrial electron transport chain

(ETC) and microsomal ETC was determined by the growth of diformazan concentration, formed in the reaction of SAR with nitro blue tetrazolium [10]. Superoxide dismutase (SOD) activity was determined by inhibition of adrenaline autooxidation, while catalase activity was determined by the amount of hydrogen peroxide, remained after its catalase-dependent reduction [6]. The concentration of free malondialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole resulting in formation of specific colored substance [24].

Statistical processing of the study results was carried out using the Microsoft Office Excel software and the Real Statistics 2019 extension to it. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between the groups. The difference was considered statistically significant at $p < 0.05$.

Results

Studying the histological preparations on the 90th day of the experiment, we established changes in the structure of both the parenchyma and the stroma of the testes. Thus, when we studied the convoluted tubules, testes, the cells were at different stages of spermatogenesis. The basement membrane was tortuous, enlarged, swollen. Disorganization of the cells of both the adluminal and basal layers of the spermatogenic epithelium was found in some places. The cells of the spermatogonia, A and B, are mostly connected to the basement membrane, but the spermatocytes of the first order are detached from them. Second-order spermatocytes and spermatids increased in size, but their number was constant compared to the control group of animals. Spermatozoa were inside the tubules, but morphologically a large number of changed cells were distinguished. Supporting cells are enlarged, swollen. The nucleus and cytoplasm of these cells are mostly illuminated (Fig. 1).

In the experimental group of animals, it was established that the tissue of the actual interstitial space of the testicles was swollen due to all components. When studying the microcirculatory bed of the tissue, it was established that the vessels were enlarged in size both due to the swollen wall of the vessels and due to an increase in their diameter. The number of vessels increased sharply, stasis was noted in them. Intravascular leukocytes inside the vessels form an areole of cells that are active before migration into the interstitial space (Fig. 2). The number of cells in the interstitial space is increased due to the migratory activity of the cells of the leukocyte pool, among which cells of the macrophage series are most often found.

Studying the cell composition of the interstitial space we found that the majority of cells are interstitial endocrinocytes. The number of cells, compared to the control group, was constant. They were clearly detected on sections where cells were located in groups from 2 to 5. These are cells with light basophilic nuclei and heterochromatin, which was clearly visualized. The cytoplasm is light, with an increased content of lipid granules. Outside of the cells, interstitial tissue was

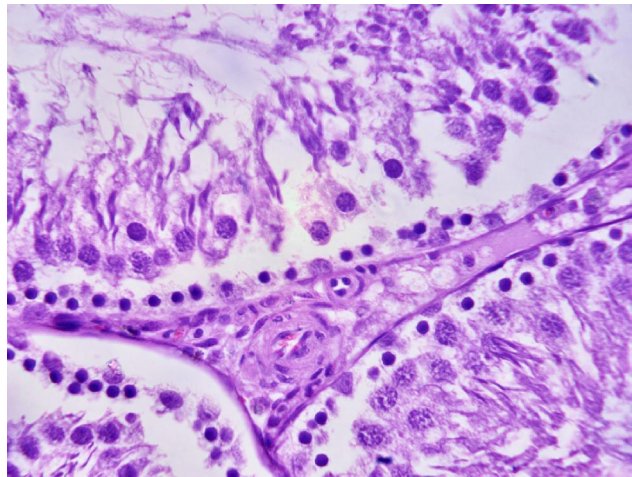


Fig. 1. Seminiferous tubules of experimental rat on the 90th day. Microimage. Stain: hematoxiline and eosine. Lens: 10; Ocular lens: 10.

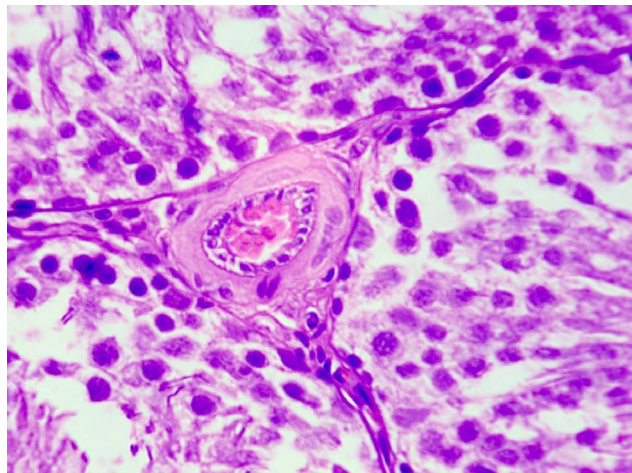


Fig. 2. Seminiferous tubules of experimental rat on the 90th day. Microimage. Interstitial space with stasis. Stain: hematoxiline and eosine. Lens: 10; Ocular lens: 10.

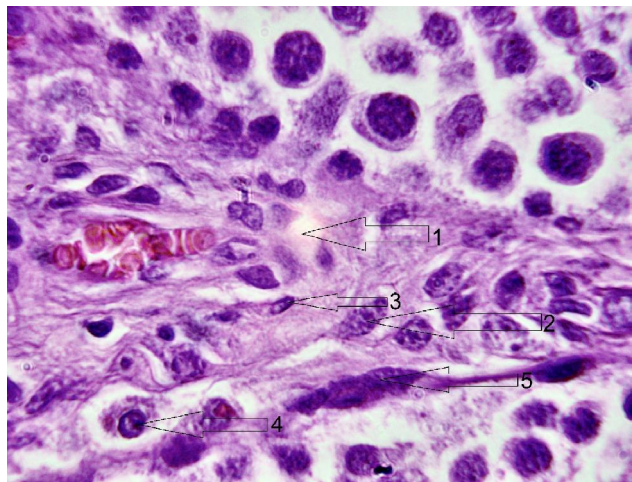


Fig. 3. Interstitial space of experimental rat on the 90th day. Microimage. 1 - interstitial space; 2 - interstitial endocrinocytes large; 3 - interstitial endocrinocytes small; 4 - interstitial macrophages; 5 - parietal macrophages. Stain: hematoxiline and eosine. Lens: 40; Ocular lens: 15.

Table 1. Markers of the NO cycle during the 90-day central blockade of LH + quercetin synthesis (M±m).

Groups	Parameters				
	gNOS activity, mol/min per g of protein	iNOS activity, mol/min per g of protein	cNOS activity, mol/min per g of protein	Arginase activity, mol/min per g of protein	NO ₂ -concentration, nmol/L
Control	0.542±0.041	0.130±0.022	0.411±0.032	2.480±0.052	3.834±0.252
Experimental	0,784±0,060*	0.639±0,061*	0,145±0.021*	0,767±0,020*	13.55±0.37*

Note: * - indicates that the difference is statistically significant when compared with control group (p<0.05).

Table 2. Markers of oxidative stress during the 90-day central blockade of synthesis of LH + quercetin (M±m).

Groups	Parameters					
	SOD activity, c.u.	Catalase activity, nkat/g of tissue	Basic O ₂ - production, nmol/s per g of tissue	Production of O ₂ - from mitochondrial ETC, nmol/s per g of tissue	Production of O ₂ - from microsomal ETC, nmol/s per g of tissue	Free MDA, nmol/g of tissue
Control	1.870±0.111	0.182±0.173	0.261±0.010	7.840±0.132	9.552±0.191	6.643±1.440
Experimental	3.812±0.181*	0.312±0.020*	2.244±0.133*	26.24±0.88*	22.38±0.29*	12.92±0.65*

Note: * - indicates that the difference is statistically significant when compared with control group (p<0.05).

lightened due to the presence of synthesis products in them. The cells usually had a polygonal shape. The established changes in cell sizes made it possible to divide the population of these cells into small and large ones by size and functional activity (Fig. 3).

Macrophages are other, and, in our opinion, the most important cells of the interstitial space of the testes, which affect the hemostasis of the connective tissue. During our study of the macrophage system of testes, several populations of these cells were found. Thus, depending on the localization in the connective tissue and the vascular bed of the testes, the following was established: parietal macrophages had an elongated flattened oval nucleus, they were located near the basal membrane of convoluted tubules, their number was increased, compared to the control group; interstitial macrophages were single and inactive (see Fig. 3), and intravascular macrophages were active before migration (see Fig. 2). Interstitial macrophages were large compared to other cell populations, their nuclei were oval or round in shape, electron dense with a predominance of heterochromatin. The cytoplasm of the cells was small in size with all subcellular elements. Intervascular macrophages morphologically resembled interstitial macrophages (see Fig. 3).

The following results were obtained as a result of the study of biochemical indicators of testicular tissues. Total NOS activity on the 90th day of central blockade of luteinizing hormone synthesis was 0.784±0.060 μmol/min, which differed from the values of the control group of animals (0.542±0.041 μmol/min) (Table 1), but not reliably. During the study of cNOS activity, indicators decreased by 2.8 times, and iNOS activity increased by 4.9 times, compared to the control group of animals. The concentration of nitrite in the testes of rats increases by 3.5 times, and the indicator of arginase activity decreases by 3.2 times.

The activity of SOD decreased by 2.0 times. The concentration of MDA in the testes of rats was reduced by 1.95 times. Basic production of O₂ - on the 90th day of the

experiment increased 8.6 times compared to the control group (Table 2). O₂ production of mitochondrial ETC decreased by 3.3 times, and microsomal ETC - by 2.3 times.

Discussion

The testicle is an immunoprivileged organ that is sensitive to oxidative stress and inflammation, two major factors that affect male infertility. It has been shown that a decrease in the concentration and activity of biomarkers of testicular function is correlated with a disturbance of the hypothalamic-pituitary-testicular axis and oxidative stress. However, the use of natural products to alleviate these changes caused by oxidative stress may be important for improving male reproductive function [8, 15].

Our study aimed to examine the effect of quercetin on triptorelin-induced oxidative stress and inflammation in the testicular interstitial space. The increase in intracellular levels of the oxidative stress system (ROS) was reduced after quercetin treatment, which was confirmed in materials with the induction of oxidative stress by lipopolysaccharide, which almost completely abolished the mRNA and protein expression of nicotinamide adenine dinucleotide phosphate oxidase 2 induced by Salmonella lipopolysaccharide (LPS) stimulation. In addition, it was confirmed in the literature that quercetin inhibited the nuclear translocation of nuclear factor kappa B (NF-κB) and reduced the levels of TNF-α, interleukin 1 (IL-1) and IL-6, which were significantly increased after exposure to LPS [13].

The indicators of our study indicate an increase in NO production in conditions of central blocking of the synthesis of luteinizing hormone, with a direct effect on interstitial endocrinocytes, to testosterone production, which is ensured by the activity of inducible iNOS. At the same time, an increase in iNOS activity with a decrease in the activity of the arginase pathway of L-arginine cleavage may indicate a change in the polarization of testicular macrophages with a preference for the pro-inflammatory phenotype (M1), which we have in the detected active parietal forms. Oxidative stress

caused by the production of reactive oxygen species plays a major role in the inflammatory processes of testes [14]. We hypothesize that modulation of oxidative stress by quercetin may protect against oxidative stress and inflammation in testicular tissue. Our data showed that quercetin reduced triptorelin-induced oxidative stress and inflammation in the testis structure as a result of inhibiting LH production. The obtained results are consistent with the literature, which describes the main points and regularities of the organization of the population of cells in the interstitial space of the testis [8].

The obtained results are a theoretical justification for the development of methods for correcting violations of the generative and endocrine function of the testes in case of extreme effects on the body, with damage to endo- and paracrine regulations. Data on the functional morphology of the testes at the stages of adaptation to changes in the endocrine and immune function of the testes expand the existing understanding of the causes of spermatogenesis disorders and its regulation. The data can be used in research work and teaching issues at departments of medical schools and biological faculties of universities.

References

- [1] Akimov, O. Y., & Kostenko, V. O. (2016). Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. *The Ukrainian Biochemical Journal*, 88(6), 70-75.
- [2] Almeida, S., Rato, L., Sousa, M., Alves, M. G., & Oliveira, P. F. (2017). Fertility and sperm quality in the aging male. *Current pharmaceutical design*, 23(30), 4429-4437. doi: 10.2174/1381612823666170503150313
- [3] Atallah, A., Mhaouty-Kodja, S., & Grange-Messent, V. (2017). Chronic depletion of gonadal testosterone leads to blood-brain barrier dysfunction and inflammation in male mice. *Journal of Cerebral Blood Flow & Metabolism*, 37(9), 3161-3175. doi: 10.1177/0271678 X16683961
- [4] Bahriy, M. M., Dibrova, V. A., Popadynets, O. H., & Hryshchuk, M. I. (2016). *Методики морфологічних досліджень: монографія [Methods of morphological research: monograph]*. Вінниця: Нова книга - Vinnytsya: Nova knyha.
- [5] Botte, M. C., Lerrant, Y., Lozach, A., Berault, A., Counin, R., & Kottler, M. L. (1999). LH down-regulates gonadotropin-releasing hormone (GnRH) receptor, but not GnRH, mRNA levels in the rat testis. *Journal of Endocrinology*, 162(3), 409-415. doi: 10.1677/joe.0.1620409
- [6] Gosalvez, J., Coppola, L., Fernandez, J. L., Lopez-Fernandez, C., Gongora, A., Faundez, R., ... & Esteves, S. C. (2017). Multi-centre assessment of nitroblue tetrazolium reactivity in human semen as a potential marker of oxidative stress. *Reproductive Biomedicine Online*, 34(5), 513-521. doi: 10.1016/j.rbmo.2017.01.014
- [7] Hotta, Y., Kataoka, T., & Kimura, K. (2019). Testosterone deficiency and endothelial dysfunction: nitric oxide, asymmetric dimethylarginine, and endothelial progenitor cells. *Sexual medicine reviews*, 7(4), 661-668. doi: 10.1016/j.sxmr.2019.02.005.
- [8] Jovicic, M., Pintus, E., Fenclova, T., Simonik, O., Chmelikova, E., Ros-Santaella, J. L., & Sedmikova, M. (2018). Effect of nitric oxide on boar sperm motility, membrane integrity, and acrosomal status during semen storage. *Polish Journal of Veterinary Sciences*, 21(1), 73-82. doi: 10.24425/119024
- [9] Ji, C., Xu, Y., Han, F., Sun, D., Zhang, H., Li, X., ... & Wang, H. (2017). Quercetin alleviates thermal and cold hyperalgesia in a rat neuropathic pain model by inhibiting Toll-like receptor signaling. *Biomedicine & Pharmacotherapy*, 94, 652-658. doi:10.1016/j.biopha.2017.07.145
- [10] Kozaeva, R., Klymenko, M. O., Katrushov, O. V., & Kostenko, V. O. (2022). Bioflavonoids as agents for correcting nitro-oxidative stress and salivary gland functions in rats exposed to alcohol during modeled lipopolysaccharide-induced systemic inflammatory response. *Wiad Lek*, 75(3), 685-690. doi: 10.36740/WLek202203121
- [11] Liu, F. H., Yang, D. Z., Wang, Y. F., Liang, X. P., Peng, W. M., Cao, C. A., ... & Guo, Z. M. (2007). Making of the animal model with sterilized testes. *Zhonghua nan ke xue= National Journal of Andrology*, 13(2), 125-129. PMID: 17345767
- [12] Merseburger, A. S., & Hupe, M. C. (2016). An update on triptorelin: current thinking on androgen deprivation therapy for prostate cancer. *Advances in therapy*, 33, 1072-1093. doi: 10.1007/s12325-016-0351-4
- [13] Mossadegh-Keller, N., & Sieweke, M. H. (2018). Testicular macrophages: Guardians of fertility. *Cellular Immunology*, 330, 120-125. doi: 10.1016/j.cellimm.2018.03.009
- [14] Morris Jr, S. M. (2016). Arginine metabolism revisited. *The Journal of Nutrition*, 146(12), 2579S-2586S. doi: 10.3945/jn.115.226621
- [15] Rawla, P. (2019). Epidemiology of prostate cancer. *World Journal of Oncology*, 10(2), 63. doi: 10.14740/wjon1191
- [16] Rice, M. A., Malhotra, S. V., & Stoyanova, T. (2019). Second-generation antiandrogens: from discovery to standard of care in castration resistant prostate cancer. *Frontiers in oncology*, 9, 801. doi: 10.3389/fonc.2019.00801
- [17] Scovell, J. M., & Khera, M. (2018). Testosterone replacement therapy versus clomiphene citrate in the young hypogonadal male. *European Urology Focus*, 4(3), 321-323. doi: 10.1016/j.euf.2018.07.033
- [18] Skrypyuk, I., Maslova, G., Lymanets, T., & Gusachenko, I.

Conclusions

1. Central blockade of LH synthesis by the administration of triptorelin with the parallel administration of quercetin to the studied animals on the 90th day of the experiment causes changes in the structure of the interstitial space of rat testes, which is characterized by high variability in the populations of interstitial endocrinocytes and macrophages.

2. Biochemical indicators on the 90th day of the experiment indicate an increase in NO production in conditions of central blocking of the synthesis of luteinizing hormone by more than three times, which is ensured by the activity of inducible iNOS. At the same time, an increase in iNOS activity with a decrease in the activity of the arginase pathway leads to the polarization of macrophages according to the pro-inflammatory type.

3. Administration of quercetin protects testicular tissue of rats from oxidative damage caused by administration of triptorelin on the 90th day of the experiment due to increased antioxidant protection and reduction of reactive oxygen species in the tissue.

- (2017). L-arginine is an effective medication for prevention of endothelial dysfunction, a predictor of anthracycline cardiotoxicity in patients with acute leukemia. *Experimental Oncology*, 39(4), 308-311. PMID: 29284775
- [19] Stetsuk, Ye. V., Kostenko, V. O., Shepitko, V. I., & Goltsev, A. N. (2019). Influence of the 30-days central deprivation of testosterone synthesis on the morphological and functional features of rat testicular interstitial endocrinocytes and sustentocytes. *World of Medicine and Biology*, 4(70), 228-233. doi: 10.26724/2079-8334-2019-4-70-228-233
- [20] Stetsuk, Ye. V., Shepitko, V. I., Akimov, O. Y., Yakushko, O. S., & Solovyova, N. V. (2021). Influence of quercetin on biochemical and morphological changes in rat testes after 30 days long central deprivation of luteinizing hormone, *World of Medicine and Biology*, 3(77), 243-248. doi: 10.26724/2079-8334-2021-3-77-243-248
- [21] Swelum, A. A. A., Saadeldin, I. M., Zaher, H. A., Alsharifi, S. A., & Alowaimer, A. N. (2017). Effect of sexual excitation on testosterone and nitric oxide levels of water buffalo bulls (*Bubalus bubalis*) with different categories of sexual behavior and their correlation with each other. *Animal Reproduction Science*, 181, 151-158. doi: 10.1016/j.anireprosci.2017.04.003
- [22] Yavtushenko, I. V., Nazarenko, S. M., Katrushov, O. V., & Kostenko, V. O. (2020). Quercetin limits the progression of oxidative and nitrosative stress in the rats' tissues after experimental traumatic brain injury. *Wiadomosci Lekarskie*, 73(10), 2127-2132. PMID: 33310934
- [23] Yang, W., Li, L., Huang, X., Kan, G., Lin, L., Cheng, J., ... & Cui, S. (2017). Levels of Leydig cell autophagy regulate the fertility of male naked mole-rats. *Oncotarget*, 8(58), 98677. doi: 10.18632/oncotarget.22088
- [24] Yelins'ka, A. M., Liashenko, L. I., & Kostenko, V. O. (2019). Quercetin potentiates antiradical properties of epigallocatechin-3-gallate in periodontium of rats under systemic and local administration of lipopolysaccharide of *Salmonella typhi*. *Wiadomosci Lekarskie* (Warsaw, Poland: 1960), 72(8), 1499-1503. PMID: 32012499
- [25] Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules*, 24(6), 1123. doi: 10.3390/molecules24061123
- [26] Zhao, Y., Liu, X., Qu, Y., Wang, L., Geng, D., Chen, W., ... & Lv, P. (2019). The roles of p38 MAPK@ COX2 and NF-kB@ COX2 signal pathways in age-related testosterone reduction. *Scientific Reports*, 9(1), 1-11. doi: 10.1038/s41598-019-46794-5

ВПЛИВ КВЕРЦЕТИНУ НА МОРФОГЕНЕЗ ІНТЕРСТИЦІЙНОГО ПРОСТОРУ В СІМ'ЯНИКАХ ЩУРІВ ЧЕРЕЗ 90 ДНІВ ПРИ ЦЕНТРАЛЬНОМУ БЛОКУВАННІ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ

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Клітини Лейдига відповідають за вироблення тестостерону в чоловічих яєчках при стимуляції лютеїнізуючим гормоном (ЛГ). Дисфункція клітин Лейдига, що виникає при запаленнях або окисних стресах, є однією з основних причин чоловічого безпліддя. Мета дослідження - встановити вплив кверцетину на мікроскопічну організацію сім'яників щурів, продукцію оксиду азоту та інтенсивність оксидативного стресу в сім'яниках щурів на 90-ту добу експерименту під час експериментальної центральної депривації синтезу ЛГ, викликаного введенням розчину триптореліна ацетату. Експеримент проводили на 20 статевозрілих самцях білих щурів. Щури були розподілені на 2 групи по 10 тварин у кожній групі: контрольна група (I), група з центральною депривацією синтезу ЛГ + кверцетин (II). Тваринам із групи з центральною блокадою синтезу ЛГ підшкірно вводили триптореліну ацетат у дозі 0,3 мг діючої речовини на кг та кверцетин 100 мг на кг маси тіла 3 рази на тиждень, при цьому контрольній групі вводили фізіологічний розчин. Проведене нами дослідження інтерстиційного простору в сім'яниках білих щурів показало неоднорідність популяцій макрофагів та мінливість структурно-функціональних параметрів. Центральна блокада синтезу ЛГ шляхом введення триптореліну з паралельним введенням кверцетину досліджуваним тваринам на 90-й день експерименту викликає зміни в структурі інтерстиціального простору сім'яників щурів, що характеризується високою варіабельністю як у популяціях інтерстиціальних ендокриноцитів, так і в макрофагах. Біохімічні показники на 90-й день експерименту вказують на підвищення продукції NO в умовах центрального блокування синтезу лютеїнізуючого гормону понад три рази, що забезпечується активністю індукцибельної ізоформи NOS (iNOS). У той же час підвищення активності iNOS при зниженні активності аргіназного шляху призводить до поляризації макрофагів за прозапальним типом. Введення кверцетину захищає тканину яєчок щурів від окисного пошкодження, викликаного введенням триптореліну, на 90-й день експерименту за рахунок підвищення антиоксидантного захисту та зниження активних форм кисню в тканині.

Ключові слова: сім'яники, інтерстиційні ендокриноцити, макрофаги, кверцетин, трипторелін, оксидативний стрес, щури.
