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Influence of Different Resveratrol Dosage Forms on Indicators of Endogenous Neuroprotection in Experimental Hypoestrogenic State

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Relevance. The aging of the female body is one of the most actual problems of modern gynecology. According to the World Health Organization (WHO) "healthy aging" was identified as one of the newest priorities in the work of national health care systems in 2014 [1]. During menopause, decreased synthesis and secretion of sex steroid hormones estrogens lead to the attenuation of functions of organs and systems [2, 3, 4]. There will be 1 billion 200 million women on earth in the menopausal period by 2030 [5].

Menopause in women is a physiological condition. During this period, only 20% of women do not experience problems [5], and more than 50% of women with reduced estrogen production have various symptoms of menopausal disorders [6]. Reduced estrogen synthesis causes diverse clinical manifestations of different time-onset. The early symptoms include vasomotor and psychoemotional disorders. Then sexual dysfunction, urogenital disorders, atrophic changes in the skin, nails, hair, and mucous membranes of the vagina appear. The most severe and dramatic consequence of the latest clinical manifestations is menopausal metabolic syndrome. It is caused by atherosclerosis, hypertension, osteoporosis, and neurological symptoms such as decreased cognitive function, memory, Alzheimer's disease [7]. The most effective method of preventing and treating estrogen

deficiency of various etiologies is hormone replacement therapy (HRT). However, long-term hormone therapy is associated with various side effects, including an increased risk of coronary heart disease, stroke, heart attack, thrombosis, and breast tumors [8, 9]. There is also a problem with patients' negative attitudes toward hormonal drugs (hormonal phobia). Therefore, the frequency of HRT in European countries is, on average, less than 30% [10].

According to the recent researches, the number of publications about alternative treatment using phytoestrogens is growing. Plant extracts containing phytoestrogens are more effective and safe for the development of adverse reactions. Phytoestrogen-containing drugs are an alternative to hormone therapy because they have estrogen-like, antioxidant, anti-inflammatory, antimicrobial, antitumor types of pharmacological action [11]. The use of plant extracts containing phytoestrogens, especially in the initial stages of menopausal syndrome, has been proven by numerous clinical trials [12, 13, 14]. To preserve and improve the quality of life of women during menopause, it is extremely important to timely diagnose menopausal disorders and begin preventive and curative therapy.

Objective. The study aimed to evaluate the neuroprotective effect of vaginal gel with resveratrol and hyaluronic acid (as monotherapy) or combining vaginal gel with resveratrol and hyaluronic acid and oral administration of resveratrol (combined administration of phytoestrogen) as the treatment regimens for

Materials and methods. Vaginal gels with resveratrol and hyaluronic acid were developed under the guidance of Doctor of Pharmacy, Professor Ruban O.A. at the Department of Industrial Technology of the National University of Pharmacy (Kharkov). Resveratrol substance contains 50% trans-resveratrol of plant origin. It was obtained from *Polygonum Cuspidatum* from the manufacturer of pharmaceutical substances "Naturex S.p. A" (France), supplier LLC "Company Euroimpex" (series №C091 / 004 / A16). In addition to phytoestrogen resveratrol, the vaginal gel contains hyaluronic and lactic acid. These substances are important components of the vaginal environment, they have antioxidant [15] and antimicrobial action, effects on wound healing, and capillary strengthening [16].

Vaginal cream "Colpotrophin" Netherlands, series: 6H772 was selected as a comparison drug. The cream contains synthetic estrogen - promestrin (P) at a dose of 10 mg/g.

The experiments were performed on 30 outbred white nonlinear female rats weighing 220-240 g and 4.5 months of age. Rats were kept in standard vivarium conditions: temperature - 20-25 ° C, relative humidity - 50-55%, natural light, diet recommended for these species, drinking regime "ad libitum". Experimental studies were conducted following the basic principles of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and others [17, 18].

The chemical substance VCD (4-vinyl cyclohexene diepoxide; 4-vinyl cyclohexene diepoxide) was used to model hypoestrogenic state in rats with intact ovaries. The VCD model is recommended for the study of disorders of the musculo-

skeletal system, cardiovascular system, and CNS in perimenopause and menopause. We used VCD (Sigma-Aldrich), which was diluted with refined corn oil and administered subcutaneously daily for 15 days at a dose of 60 mg/kg. This dose destroys 80-90% of small preantral (primordial and primary) follicles in the ovaries of female rats, accelerating the processes of atresia and thereby causing premature development of perimenopause/menopause [19]. Vaginal gel with resveratrol (R) and hyaluronic acid (HA) (for monotherapy), vaginal gel with resveratrol (R) and hyaluronic acid and resveratrol tablets (iHerb, USA) (for combination therapy), and the reference drug cream "Colpotrophin" were administered intravaginally after 15 days of chemical stimulation of hypoestrogenic state. The duration of treatment with vaginal gels was 28 days. Gel with resveratrol and hyaluronic acid and cream with P were administered vaginally once a day using a syringe-dispenser with an atraumatic tip of 0.005 ml/kg. Tablets with resveratrol at a dose of 100 mg/kg were transformed into suspension and administered intragastrically using a gavage.

Rats' biological material was taken under thiopental anesthesia (40 mg/kg). Blood from the abdominal artery and brain were used for research. Blood was rapidly removed from the brain, separated from the meninges, and the test pieces were placed in liquid nitrogen. Then ground in liquid nitrogen to a powdery state and homogenized in a 10-fold volume of a medium at (2 ° C) containing (in mmol): sucrose - 250, Tris-HCl buffer - 20, EDTA - 1 (pH 7.4). Mitochondrial fraction was isolated by differential centrifugation on a Sigma 3-30k refrigerator centrifuge (Germany) at a temperature (+ 4 ° C). The brain of the experimental animals was placed for a day in Buen's fixator and after standard histological conduction, the tissue was placed in a paraplast. Sections of CA-1 hippocampal zone with a thickness of 5 microns were made for PCR on a rotating microtome.

Real-time PCR

The expression pattern of HSP70, HIF-1, c-fos mRNA was determined by real-time polymerase chain reaction. The tissues were dewaxed by incubation in xylenes and 100% ethanol baths. After dewaxing and centrifugation, the precipitate was dried in air to remove ethanol residues.

Isolation of total RNA from rat tissue was performed using the kit "Trizol RNA Prep 100" (ISOGEN ", Russia). This kit contains the following reagents: Trizol reagent and ExtraGene E. RNA was isolated according to the protocol of the kit. "Set of reagents for reverse transcription (OT-1)" ("syntola", Moscow) was used for reverse transcription (synthesis to DNA). Amplifier CFX96™ Real-Time PCR Detection Systems ("Bio-Rad Laboratories, Inc.", USA) and a set of reagents for PCR-RV in the presence of SYBR Green R-402 ("Sintol", Russia) were used to determine the expression level of the studied genes. For analysis of studied and reference genes, specific primer pairs (5'-3' ') were selected using PrimerBlast software (www.ncbi.nlm.nih.gov/tools/primer-blast). They were manufactured by ThermoScientific, USA. The fluorescence intensity was recorded automatically at the end of the elongation stage of each cycle on the SybrGreen channel. The actin, beta (Actb) gene was used as a reference gene to determine the relative value of the change in the expression level of the studied genes.

Enzyme-linked immunosorbent assay (ELISA)

The principle of the method is based on solid-phase enzyme-linked immunoadsorption, on the principle of "sandwich". The level of HSP70 heat shock protein was determined in the cytoplasmic and mitochondrial fractions of the brain by enzyme-linked immunosorbent assay using the AMP'D® HSP70 high sensitivity ELISA kit, Enzo (Sweden). The concentration of HSP70 was expressed in ng/ml.

Statistics

The data are presented as the arithmetic mean and standard error of the mean value ($M \pm m$). The results of the study were processed using the statistical package of the licensed program STATISTICA® for Windows 6.0 (StatSoftInc., № AXXR712D833214FAN5), as well as "Microsoft Excel 2010". Statistical processing was performed using Student's t-test and Mann-Whitney U-test. Differences with a significance level less than 0.05 (95%) were considered statistically significant for all types of analysis [20].

Research results

Table 1 characterizes the expression of HIF-1 α , HSP70 mRNA, and c-fos mRNA in the hippocampal CA1 region of female rats' brains with VCD stimulated hypoestrogenic state. Expression of HIF-1 α mRNA in the untreated rat group (control) was 92.2% lower compared to the intact group. There was also a decrease in the expression of HSP70 mRNA by 92.5% and c-fos mRNA by 80.6%. Enzyme-linked immunosorbent assay revealed a decrease in the concentration of HSP70 in mitochondria by 61% and the cytosol of the brain of female rats with VCD stimulated hypoestrogenic state compared with healthy females of the same age.

Course treatment using vaginal cream with P did not significantly affect the expression of HIF-1 α mRNA, HSP70 mRNA, and c-fos mRNA, as well as the concentration of HSP70 in the brains of female rats with VCD stimulated hypoestrogenic state. Course administration of vaginal gel containing R and HA to female rats with VCD stimulated hypoestrogenic state led to a significant increase in the expression of HIF-1 α mRNA (150%), HSP70 mRNA (33%), and c-fos mRNA (200%) in hippocampal CA1 region.

Also, administration of the vaginal gel with R and HA led to a significant increase in the concentration of HSP70 by 20% in the cytosolic fractions of the brain of female rats with the hypoestrogenic state. Course combined usage of gel and tablets with resveratrol led to a significant increase in the expression of HIF-1 α mRNA, HSP70 mRNA, and c-fos mRNA in the hippocampal CA1 region of females with hypoestrogenic state 23 times, 30.7 times, and 6.7 times, respectively, citing these indicators to IC values or increasing them by 2.3 times (HSP70 mRNA). Combined administration of vaginal gel and tablets with resveratrol to female rats with VCD stimulated hypoestrogenic state significantly increased the concentration of HSP70 in the cytosolic and mitochondrial fractions of the brain by 118 and 177 %%, respectively. The combined effect of gel and tablets with resveratrol signifi-

icantly exceeds the monotherapy of gel with R and cream with P in terms of the degree of their influence on the research parameters.

Table 1. The expression of HIF-1 α mRNA, HSP₇₀ mRNA, and c-fos mRNA in the brains of female rats with VCD stimulated hypoestrogenic state and pharmacological correction on the 29th day after treatment

Experimental group (M \pm m)	mRNA HIF-1 α	% in relation to IC	HSP ₇₀ mRNA	% in relation to IC	mRNA c-fos	% in relation to IC
IC	1,000 \pm 0,00211	–	1.000 \pm 0,00166	–	1.000 \pm 0,00830	–
CP	0,07790 \pm 0,00024	-92,21%	0,0744 \pm 0,00011	-92,56%	0,19345 \pm 0,00690	-80,66%
CP + gel with R and HA	0,19447 \pm 0,01022*	149,64%	0,09895 \pm 0,00029*	33%	0,57921 \pm 0,00192*	199,41%
CP + gel with R and HA and tablets with R	1,82738 \pm 0,00486* ^{1,2}	2246%	2,36338 \pm 0,00121* ^{1,2}	3076%	1,30514 \pm 0,03465* ^{1,2}	574,67%
CP + cream with P	0,08337 \pm 0,00382	6,94%	0,06123 \pm 0,00012	-17,7%	0,24084 \pm 0,03892	24,5%

Table 2. The concentration of HSP₇₀ in the cytoplasm and mitochondria in the brains of female rats with VCD stimulated hypoestrogenic state and pharmacological correction on the 29th day after treatment

Experimental group (M \pm m)	HSP ₇₀ , pg/ml fractions			
	Mitochondrial fraction		Cytosolic fraction	
IC	1,340 \pm 0,099	% in relation to IC	3,589 \pm 0,051	% in relation to IC
CP	0,512 \pm 0,013	-61,79	1,29 \pm 0,052	-64,06
CP + gel with R and HA	0,608 \pm 0,016	18,75	1,548 \pm 0,027*	20

Table 2 (continued). The concentration of HSP₇₀ in the cytoplasm and mitochondria in the brains of female rats with VCD stimulated hypoestrogenic state and pharmacological correction on the 29th day after treatment

CP + gel with R and HA and tablets with R	1,268± 0,039* ^{1,2}	147,66	2,821± 0,031* ^{1,2}	118,68
CP + cream with P	0,544± 0,019	6,25	1,517± 0,071	17,6

Note: * - $p < 0,05$ % in relation to controlled pathology (CP)

1 - $p < 0,05$ in relation to CP + cream with P

2 - $p < 0,05$ in relation to CP + gel with R

Discussion of results

VCD stimulated hypoestrogenic state leads to a significant deficiency of progesterone and estradiol and an increased level of FSH [21]. Estradiol and, especially, progesterone play an important role in the mechanisms of endogenous cytoprotection and neuroprotection, regulate learning, memory, psycho-emotional behavior [22]. VCD stimulated hypoestrogenic state leads to impaired expression of mRNA, and in particular mRNA encoding the superoxide dismutase and glutathione reductase gene. A decrease in the level of reduced glutathione and increased expression of iNOS, activation of oxidative stress were found in experimental animals. Our research does not contradict these conclusions. Thus, a decrease in the expression of c-fos mRNA indicates the suppression of transcriptional activity in the hippocampal neurons of female rats with VCD stimulated hypoestrogenic state.

It is known that transcription factors (c-fos and c-jun) play a significant role in the initiation of the endogenous neuroprotection mechanisms. These factors trigger the expression of heat shock protein genes (HSP70 and HSP72), as well as factor induced by hypoxia (HIF-1). c-fos play a crucial role in controlling the cell cycle, development, growth, and cell differentiation and function, they are involved in the transmission of information from cell to cell. When the adaptive capacity of the organism is disrupted, ROS level is increased and the level of reduced thiols is decreased, transcription factors are inactivated with subsequent inhibition of c-fos gene expression [24]. The detected decrease in HSP70 mRNA expression and HSP70 concentration in the brain of rats with VCD stimulated hypoestrogenic state may be due to both progesterone and glutathione deficiency.

It is known that aggregation and accumulation of denatured proteins, activation of free radical processes, and calcium overload are the ischemic cellular signs. HSP70s can limit the accumulation of denatured proteins, activate free radical processes, increase the potency of antioxidant enzymes and limit the damaging effects of calcium overload by binding to the calcium receptor-calmodulin, as well as limiting the expression of iNOS [25]. Experiments showed the ability of HSP70 to prolong the lifetime of the main adaptation factor - HIF-1.

In extreme conditions, this factor initiates the launch of compensatory mechanisms of energy production [26]. Thus, an experimental hypoestrogenic state leads to a significant deprivation of the endogenous neuroprotection mechanisms associated with HSP70. The revealed neuroprotective properties of resveratrol, especially combining its dosage forms - vaginal gel and tablets, can be explained as follows. It is known that estrogens, including phytoestrogens, are able to simulate the expression of global transcription factors responsible for the synthesis of HSP proteins. In addition, the activation of the modulation of β -estrogen receptors in the brain causes the separation of the last HSP70 - proteins, which ensures the entry of these proteins into the cell and the realization of their biological function. The mechanism of this interaction is related to the role of HSP70 in maintaining inactive estrogen receptors not associated with estrogen [26]. It is assumed that binding of the receptor with the steroid ligand leads to conformational changes in the receptor molecule, its release from the complex with heat shock proteins, and their entry into the cell.

Bioflavonoids in resveratrol can increase the activity of estrogen receptors, reduced by oxidative stress, due to ROS/SH-regulation [26]. Estrogen receptor modulators can enhance HSP70 expression by stimulating the effect of protein transcription factor - heat shock factor (FHS) [26]. Estrogen modulators can inhibit neuroapoptosis by reducing the activity of caspase-3 and two nuclear factor- κ B transcription factors (p65 / RelA and p50), regulating the expression of the c-fos early response gene. Resveratrol can also act as a direct antioxidant because it has a phenolic group and can trap reactive oxygen species and nitrogen and thus regulate the JNK (ROS-sensitive) cascade. JNK activates DNA-binding proteins cJun, c-Fos, and AP-1, and binding of these proteins to palindromic DNA sequences induces apoptosis [26].

Conclusions

1. We were the first who noticed that the simulation of hypoestrogenic state in female rats by 15-day administration of VCD leads to a sharp suppression of endogenous neuroprotection associated with HSP70.

2. Evaluation of the neuroprotective effect of a 28-day course of vaginal gel with resveratrol and hyaluronic acid, vaginal cream with P, as well as a combination of gel and tablets with resveratrol in females with hypoestrogenic state, revealed the desired effect only in dosage forms of resveratrol. The most pronounced effect was observed with the combined use of vaginal gel and tablets with resveratrol.

3. Resveratrol neuroprotective action is explained by its ability to activate HSP70-mechanisms of endogenous neuroprotection, depressed by estrogen deficiency. Resveratrol increases the expression of HIF-1 α mRNA, HSP70 mRNA, and c-fos mRNA, as well as the concentration of HSP70 in the brain.

4. The obtained results confirm the expediency of developing a new vaginal gel with resveratrol and hyaluronic acid as an alternative to hormone-containing drugs. These substances can be used for the prevention and treatment of pathological hypoestrogenic conditions that occur on the background of estrogen deficiency.

References

- [1] Здоровое старение должно стать глобальным приоритетом. Выпуск новостей, ВОЗ. 06.11.2014. "Ageing well" must be a global priority 6 November 2014 News release GENEVA Reading time: 3 min (709 words)
<https://www.who.int/ru/news/item/06-11-2014--ageing-well-must-be-a-global-priority>
- [2] Иловайская И.А., Войташевский К.В. Менопаузальная гормональная терапия: возможности и риски, StatusPraesens. Гинекология. Акушерство. Бесплодный брак. 2015. № 5 (28). С. 80–86.
<https://cyberleninka.ru/article/n/klimaktericheskiy-sindrom-sovremennoe-sostoyanie-voprosa>
- CLIMACTERIC SYNDROME: CURRENT STATUS OF THE ISSUE
(LITERATURE REVIEW) Dvoryanskiy S.A., Emelyanova D.I., Yagovkina N.V.
- [3] Хамошина М.Б., Роговская С.И., Наумчик Г.А. Актуальные возможности фармакологии в профилактике возрастных нарушений, StatusPraesens. Гинекология. Акушерство. Бесплодный брак. 2014. № 1 (18). С. 75–81.
<https://journaldoctor.ru/upload/iblock/9dd/1.pdf>
- Unsolved Problems in Modern Gynecology: Quo vadis? V. E. Radzinskiy, M. B. Khamoshina Peoples' Friendship University of Russia, Moscow
- [4] Шестакова И.Г., Беттихер О.А., Алеев И.А. Урогенитальная атрофия как следствие дефицита эстрогенов: излечима и предотвратима, StatusPraesens. Гинекология. Акушерство. Бесплодный брак. 2015. № 5 (28). С. 52–59.
https://umedp.ru/articles/besplodnyu_brak_algoritmy_diagnostics_i_lecheniya.html
- [5] Плаксина Н.Д., Симоновская Х.Ю. Возможности негормональной коррекции вазомоторных пароксизмов в постменопаузе, StatusPraesens. Гинекология. Акушерство. Бесплодный брак. 2014. № 2 (19). С. 60–65.
http://vyatmedvestnik.ru/index.php/vmv/issue/download/4/no1_53_2017
- [6] Сметник В.П. Старение репродуктивной системы женщины: клинко-гормональное обоснование стадий, терминология, Доктор.ру. Гинекология. Эндокринология. – 2014. № 12 (100). С. 13–16.
<https://journal.gynecology.school/wp-content/uploads/2019/02/Starenie-reproduktivnoj-sistemy-zhenshhiny.pdf>
- Female Reproductive Aging: Describing Terminology and Identifying Different Stages Based on Clinical and Hormonal Characteristics V. P. Smetnik Academician V. I. Kulakov Research Center for Obstetrics, Gynecology and Perinatology, Moscow
- [7] Менопаузальная гормональная терапия и сохранение здоровья женщин зрелого возраста: Клинические рекомендации (протокол лечения). М.: ФГБУ НЦГАиП, 2015. 49 с.
<https://minzdrav.gov-murman.ru/activities/akusherstvo/poryadki/menopauza.pdf>

- [8] Dinger J., Mhoner S., Heinemann K. Cardiovascular risks associated with the use of drospirenone-containing combined oral contraceptives, *Contraception*. 2016. Vol. 93, N 5. P. 378–385. <https://pubmed.ncbi.nlm.nih.gov/26825258/>
- [9] Roach R.E.J., Helmerhorst F.M., Lijfering W.M. et al. The risk of heart attack and stroke in women using birth control pills, *Cochrane Database Syst. Rev.* 2015 July 8: CD011054. https://www.cochrane.org/CD011054/FERTILREG_risk-heart-attack-and-stroke-women-using-birth-control-pills
- [10] Зайченко Г. В., Карпенко Н. О., Стрига О. А., Сініцина О. С. Фармакологічний менеджмент менопаузальних розладів: фітоестрогени (огляд літератури), *Проблеми ендокринної патології* – 2018 – №4 – С. 65-74. http://www.irbis-nbu.gov.ua/cgi-bin/irbis_nbu/cgiirbis_64.exe?I21DBN=LINK&P21DBN=UJRN&Z21ID=&S21REF=10&S21CNR=20&S21STN=1&S21FMT=ASP_meta&C21COM=S&2_S21P03=FILA=&2_S21STR=pep_2018_4_9
PHARMACOLOGICAL MANAGEMENT OF MENOPAUSAL DISORDERS: THE PHYTOESTROGENES (review) G. V. Zaychenko, N. O. Karpenko, E. A. Striga, O. S. Sinitsyna.
- [11] Xue Y.Q. Resveratrol oligomers for the prevention and treatment of cancers / Y.Q. Xue, J.M. Di, Y. Luo [et al], *Oxid Med Cell Longev.* – 2014. – doi: 10.1155/2014/765832. <https://www.hindawi.com/journals/omcl/2014/765832/>
- [12] Rietjens IMCM, Lousse J., Beekmann K. The potential health effects of dietary phytoestrogens. *Br J Pharmacol.* 2017; 174 (11): 1263–1280. <https://doi.org/10.1111/bph.13622>, <https://pubmed.ncbi.nlm.nih.gov/27723080/>
- [13] Marija Glisic, Natyra Kastrati, Valentina Gonzalez-Jaramillo et al. Associations between Phytoestrogens, Glucose Homeostasis, and Risk of Diabetes in Women: A Systematic Review and Meta-Analysis. *Adv Nutr.* 2018, 9(6): 726–740. <https://doi.org/10.1093/advances/nmy048>
- [14] Yunxia Li, Jinbing Huang, Yuan Yan et al. Preventative effects of resveratrol and estradiol on streptozotocin-induced diabetes in ovariectomized mice and the related mechanisms. *PLoS One.* 2018; 13(10): e0204499. <https://doi.org/10.1371/journal.pone.0204499>. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6166971/>
- [15] Bavaresco I. Role of vitacultural factors on stilbene concentration of grapes and wine / I. Bavaresco, *Drugs. Exp. Clin. Res.* – 2003. – №29. – P.181-187. <http://library.um.edu.mo/ebooks/b28045580.pdf>
- [16] Фармакологічне обґрунтування розробки нових лікарських препаратів на основі ресвератролу, Г. В. Зайченко, Н. О. Горчакова, О. А. Стрига, О. І. Рубан, *Вісник проблем біології і медицини.* – 2017. – Вип. 4, Т. 1 (139). – С. 21-30. [http://www.irbis-nbu.gov.ua/cgi-bin/irbis_nbu/cgiirbis_64.exe?I21DBN=LINK&P21DBN=UJRN&Z21ID=&S21REF=10&S21CNR=20&S21STN=1&S21FMT=ASP_meta&C21COM=S&2_S21P03=FILA=&2_S21STR=Vpbm_2017_4\(1\)_8](http://www.irbis-nbu.gov.ua/cgi-bin/irbis_nbu/cgiirbis_64.exe?I21DBN=LINK&P21DBN=UJRN&Z21ID=&S21REF=10&S21CNR=20&S21STN=1&S21FMT=ASP_meta&C21COM=S&2_S21P03=FILA=&2_S21STR=Vpbm_2017_4(1)_8)

PHARMACOLOGICAL GROUND OF THE NEW DRUGS' ELABORATION ON THE RESVERATROL BASE Zaichenko A. V., Gorchakova N. A., Striga E. A., Ruban O. I.

[17] Етика лікаря та права людини: положення про використання тварин у біомедичних дослідках // Експериментальна та клінічна фізіологія і біохімія. – 2003. – № 2 (22). – С. 108–109. <https://files.odmu.edu.ua/lekcii/bioetika.pdf>

Dynamics of systems POL-AOP, carbohydrate and lipid metabolism in blood of experimental rats in transition of acute pancreatitis in chronic form V. A. Makarchuk Institute of Gastroenterology of NAMS of Ukraine, Dnipropetrovsk G. O. Ushakova Oles Honchar Dniropetrovsk National University.

[18] Закон України. Про захист тварин від жорстокого поводження [Електронний ресурс], Відомості Верховної Ради України (ВВР). – 2006. – Режим доступу: [https://vpbm.com.ua/vyipusk-1-\(135\),-2017/8649](https://vpbm.com.ua/vyipusk-1-(135),-2017/8649)

[19] Protective effect of alpha lipoic acid on 4-vinylcyclohexene diepoxide induced primary ovarian failure in female rats/Feuyaz Özel, Müge Kiray, Aslı Göker, Selma Aydemir, Serap Cilaker Mıcılı, Taiwanese Journal of Obstetrics and Gynecology.-2020.-Vol. 59, № 2.- P. 293-300.

[20] Посібник з біостатистики. Аналіз результатів медичних досліджень у пакеті EZR (R-statistics), В. Г. Гур'янов, Ю. Є. Лях, В. Д. Парій, О. В. Короткий, О. В. Чалий, К. О. Чалий, Я. В. Цехмістер: Навчальний посібник. – К. Вістка, 2018. – 208 с.

https://www.researchgate.net/profile/Yaroslav_Tsekhmister/publication/326271449_Posibnik_z_biostatistiki_analiz_rezultativ_medicnih_doslidzen_u_paketi_EZR_R-STATISTICS/links/5b436d09aca2728a0d664341/Posibnik-z-biostatistiki-analiz-rezultativ-medicnih-doslidzen-u-paketi-EZR-R-STATISTICS.pdf

[21] Abolaji, A. O., Omozokpia, M. U., Oluwamuyide, O. J., Akintola, T. E., & Farombi, E. O. Rescue role of hesperidin in 4-vinylcyclohexene diepoxide-induced toxicity in the brain, ovary and uterus of wistar rats, Journal of Basic and Clinical Physiology and Pharmacology.-2020.-Vol. 31(2).- 20180115.

<https://doi.org/10.1515/jbcpp-2018-0115>

[22] Анищенко А.М. Фитоэстрогены как альтернатива заместительной гормональной терапии при гипоестрогенемии (экспериментальное исследование).- Дисс. д.мед.н.-Томск, 2014.-227с.

[23] Biochemical Aspects of the Neuroprotective Effects of the Selective Estrogen Recertor Modulator Tamoxifen in a Model of Acute Cerebral Ischemia/ Belenichev I.F.,Pavlov S.V., Neurochemical Journal.- 2014.-Vol.8,№1.-P 28-32

[24] The neuroprotective activity of tamoxifen and tibolone during glutathione depletion in vitro/I.Belenichev, S.Pavlov, O. Odnokoz, O.Belenicheva, E.Polyakova/Neurochemical Journal .-2012.-Volume 6, Issue 3 , pp 202-212

[25] Тиол-дисульфидная система: роль в эндогенной цито- и органопротекции, пути фармакологической модуляции/Беленичев И.Ф., Чекман И.С., Нагорная Е.А., Горбачева С.В., Горчакова Н.А., Бухтиярова Н.В.- Киев: ТОВ "Видавництво "Юстон", 2020.-232с.

[26] Нейропротекция и нейропластичность/Беленичев И.Ф., Черний В.И., Бухтиярова Н.В., Горчакова Н.А., Нагорная Е.А.- Киев:Логос.-2015.-512 с.

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