UDC:616-06:616-092.9

Research of changes in the level of vascular endothelial growth factor in experimental diabetic retinopathy. comparison of methods of correction

Ya. V. Sirman, I. V. Savytskyi

GE «Ukrainian Research Institute of Transport Medicine of the Ministry of Health of Ukraine»

Correspondence author: *Savytskyi Ivan Volodymyrovich, 65039, Odesa, Fountain road 4-a/29, tel.+38050-381-21-83, e-mail-farmakod@ukr.net*

Abstract

The aim of work to investigate the changes in the vascular endothelium growth factor in experimental animals, which were simulated diabetic retinopathy and against the background of its correction.

The results of the analysis of the vascular growth factor in the 2nd group confirm the development of diabetic retinopathy, a particularly pronounced increase in the marker was detected on the 180th day of the development of the pathological process. It was established analyzing the data of group No. 3 that the correction of the pathological condition with hypoglycemic agents has partial positive effect but requires the involvement of additional means of correction in addition to the normalization of the level of glycemia. The results of the 4th group indicate that the involvement of a nitric oxide donor and aflibercept in the correction of diabetic retinopathy has positive effect on the reduction of the level of vascular

growth factor, more pronounced compared to the 3rd group, but it does not reach normative values. It is observed that the correction of the simulated pathological condition by reducing hyperglycemia, administration of aflibercept and bromfenac (group No. 5) gives positive results, but less pronounced, compared to the data of the 4th group.

It was found that in rats in which diabetic retinopathy was modeled with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac (group No. 6) at the first stage, there is a pronounced effectiveness of the proposed method of correction in comparison with the previously considered methods, the established positive trend is being followed also at the 2nd and 3rd stages of the experiment, which indicates the effectiveness of this correction method.

The most pronounced positive effect of normalizing the level of vascular growth factor is observed when using a hypoglycemic drug in combination with the administration of aflibercept, a solution of L-arginine and citicoline (in group No. 7).

Keywords: experimental diabetic retinopathy; streptozotocin diabetes; vascular growth factor; correction; metformin; aflibercept; bromfenac; L-carnitine; L-arginine; citicoline.

Introduction. According to WHO data, diabetic retinopathy is the main cause of vision loss and blindness in diabetes [1-4]

It has been proven that VEGF in blood serum is informative as a marker of the development of diabetic retinopathy [5]. In the retina under the influence of VEGF under conditions of hypoxia and with the participation of proteases, the initial stages of angiogenesis occur migration of endothelial cells in the extracellular matrix and degradation of the basal membrane of the capillary endothelium. VEGF regulates the development of newly formed vessels to the stage of involvement of pericytes, which stabilize the vascular network [6, 7]. Also, the vascular growth factor stimulates increased permeability of the vascular wall, disruption of the hematoretinal barrier functions due to the phosphorylation of tight junctions of endotheliocytes [8]. An increase in the permeability of the hematoretinal barrier leads to the development of diabetic macular edema. VEGF causes increased expression of leukocyte adhesion molecules VCAM1, ECAM1, PECAM-1, P-selectin, which in turn increase leukocyte adhesion in retinal microvessels. As a result, there is a violation of the permeability of the hematoretinal barrier, loss of endotheliocytes, infiltration of the retina with leukocytes, and diapedesis [6, 9].

A network of endotheliocytes, which is formed during vasculogenesis, serves as a framework for angiogenesis [10-12]. Inadequate angiogenesis is the basis of such a pathological process as proliferative diabetic retinopathy. The causes of a progressive decrease in visual acuity in patients with diabetes are the excessive proliferation of vessels in the eyeball. An imbalance between inhibitors and stimulators of angiogenesis is characteristic of the development of diabetic retinopathy. During retinal ischemia, hyperproduction of VEGF increases, which plays a key role in the activation of pathological neoplasms [10, 13].

The aim of work – to investigate the changes in the vascular endothelium growth factor in experimental animals, which were simulated diabetic retinopathy and against the background of its correction.

The study was conducted on white Wistar rats weighing 180-200 g. According to the tasks, the animals were divided into 7 groups:

1st group – intact animals;

2nd group – 60 animals with modelling of DR without correction (control pathology);

3rd group –60 animals with modelling of DR with correction of hyperglycemia;

4th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept and L-arginine solution;

5th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept and bromfenak;

6th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept, L-carnitine solution and bromfenak;

7th group – 60 animals with modelling of DR with correction of hyperglycemia, administration of aflibercept, L-arginine solution and citicoline.

Type 2 diabetes and DR were modeled by intraperitoneal administration of streptozotocin (Sigma, USA) dissolved in 0.1 M citrate buffer with pH 4.5 [14, 15]. Dose of streptozocin of 55 mg/kg of animal weight was divided into two administrations. Administration of streptozocin was preceded by a high-fat diet for 28 days [10].

Animals were subjected to research by decapitation in accordance with the "Rules for the performance of work using experimental animals", approved by the Order of the Ministry of Health of Ukraine No. 249 of 01.03.2012 and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (as amended on 15.12.2009 and 16.10.2012).

The hypoglycemic medicine – metformin (Merck Sante, manufactured in France) - at at a dose of 300 mg/kg body weight [11] in a 0.9 % sodium chloride solution through a syringe with an intragastric probe daily, during the entire experiment.

The introduction of L-arginine solution, which is NO donor, (SIMESTA, manufactured in China, quality standard USP32) was carried out by intragastric administration of L-arginine solution in 0.9 % sodium chloride solution at a dose of 500 mg/kg [12] through a syringe with an intragastric probe.

Aflibercept (anti-VEGF therapy) was administered in the form of subconjunctival injections at a dose of 0.08 ml (25 mg/ml) [13] with an interval of 1 injection every 30 days.

Citicoline – 81.8 mg/kg (0.33 ml/kg) was administered intramuscularly once per a day [14].

Bromfenak – was introduced of 0,09 % eyes drop solution once per a day [15].

L-carnitine (manufacturing by "Sigma", USA) was administrated in the form of an aqueous solution through a syringe with an intragastric probe at a dose of 25 mg/100 g of animal weight [16, 17].

The level of vascular endothelial growth factor (VEGF) in the blood serum of rats was determined by the immunoenzymatic method with the sets of reagents manufacturing by company "Vector BEST" [24].

To identify changes in the studied indicators between different groups and at different stages, we used parametric statistical methods, which are based on operating with the parameters of the statistical distribution (mean and variance). The methods used are designed for normally distributed data, so we checked all data for normality using E.I. Pustilnyk's asymmetry and kurtosis criterion. All the data that we are considering turned out to be normally distributed, so we can pairwise compare the mean values of the samples. Note that in the following comparisons we perform comparisons in independent samples. These will be comparisons between different groups of animals or comparisons between the same group of animals (but since there is no correspondence between animals in the samples, they will also be independent). The value $p < 0.05$ was chosen as the reliability criterion. An analysis was performed to see if the means differed. The results of determining the t-test give an answer about the equality or difference of the mean values, but they do not provide an opportunity to accurately measure the difference between the mean values. Note that this difference is quite conditional. This difference was calculated as a percentage. Thus, we demonstrated a comparison of mean values between different groups of animals.

Results of study and their discussion:

The dynamics of the VEGF in the blood serum of experimental animals with simulated diabetic retinopathy and under the influence of various methods of its correction at each stage of the experiment are presented in Table 1.

Таблиця 1 – The VEGF level in the blood of experimental animals with modelling diabetic retinopathy and its different methods of correction on the 30th, 60th and 180th day (M±m), (μM/l)

Stages / Groups	I stage(A)		2 stage (B)		3 stage (C)	
Group 1	28,9±0,44		30,45±0,5		30,19±0,51	
	-	-	1A-1B	p<0,05	1A-1C 1B-1C	p>0,05 p>0,05
Group 2	48,5±0,4		67,32±0,42		73,94±0,38	
	1A-2A	p<0,001	1B-2B 2A-2B	p<0,001 p<0,001	1C-2C 2A-2C 2B-2C	p<0,001 p<0,001 p<0,001
Group 3	42,93±0,5		50,75±0,42		54,12±0,43	
	1A-3A 2A-3A	p<0,001 p<0,001	1B-3B 2B-3B 3A-3B	p<0,001 p<0,001 p<0,001	1C-3C 2C-3C 3A-3C 3B-3C	p<0,001 p<0,001 p<0,001 p<0,001
Group 4	39,46±0,32		37,97±0,34		34,48±0,43	
	1A-4A 2A-4A 3A-4A	p<0,001 p<0,001 p<0,001	1B-4B 2B-4B 3B-4B 4A-4B	p<0,001 p<0,001 p<0,001 p<0,01	1C-4C 2C-4C 3C-4C 4A-4C 4B-4C	p<0,001 p<0,001 p<0,001 p<0,001 p<0,001
Group 5	41,27±0,43		40,65±0,61		38,92±0,53	
	1A-5A 2A-5A 3A-5A 4A-5A	p<0,001 p<0,001 p<0,05 p<0,01	1B-5B 2B-5B 3B-5B 4B-5B 5A-5B	p<0,001 p<0,001 p<0,001 p<0,001 p>0,05	1C-5C 2C-5C 3C-5C 4C-5C 5A-5C 5B-5C	p<0,001 p<0,001 p<0,001 p<0,001 p<0,01 p<0,05
Group 6	38,28±0,48		36,67±0,46		35,29±0,43	
	1A-6A 2A-6A 3A-6A 4A-6A 5A-6A	p<0,001 p<0,001 p<0,001 p<0,05 p<0,001	1B-6B 2B-6B 3B-6B 4B-6B 5B-6B 6A-6B	p<0,001 p<0,001 p<0,001 p<0,05 p<0,001 p<0,05	1C-6C 2C-6C 3C-6C 4C-6C 5C-6C 6A-6C 6B-6C	p<0,001 p<0,001 p<0,001 p>0,05 p<0,001 p<0,001 p<0,05
Group 7	33,52±0,43		31,65±0,41		30,45±0,52	
	1A-7A 2A-7A 3A-7A 4A-7A 5A-7A 6A-7A	p<0,001 p<0,001 p<0,001 p<0,001 p<0,001 p<0,001	1B-7B 2B-7B 3B-7B 4B-7B 5B-7B 6B-7B 7A-7B	p>0,05 p<0,001 p<0,001 p<0,001 p<0,001 p<0,001 p<0,01	1C-7C 2C-7C 3C-7C 4C-7C 5C-7C 6C-7C 7A-7C 7B-7C	p>0,05 p<0,001 p<0,001 p<0,001 p<0,001 p<0,001 p<0,001 p>0,05

At the 1st stage in the second group (which diabetic retinopathy was modeled without correction) was detected an increase in the VEGF level compared to the control group on 67.8 % (p<0.001). At the second stage, an increase in the studied marker was found on 121 % compared to the intact group, and on 38.8 % (p<0.001) compared to its group at the previous stage. At the third stage, it was established that the level of VEGF was on 144.9 % (p<0.001) higher compared to the intact group, on 52.4 % (p<0.001) higher than the value on the 1st stage group, and on 9.8 % (p<0.001) – of the 2nd stage.

It was established in group No. 3 (which simulated diabetic retinopathy with correction of hypoglycemia) that at the first stage, the level of the studied indicator was on 48.5 % higher than the results of the control group, and compared to group No. 2, it was lower on 11.5 %. In the second stage, an increase in VEGF is observed, its level is higher both compared to the intact group – on 66.7 % (p<0.001) and compared to the previous stage – on 18.2 % (p<0.001), compared to group 2 it is lower on 24.6 % (p<0.001). At the third stage, a more pronounced increase in VEGF compared to the intact group was established – on 79.2 % (p<0.001). It was compared to the data of its group of the first stage higher on 26 % (p<0.001), of the second stage – on 6.6 %, but compared to group No. 2, its increase is less pronounced on 26.8 % (p<0.001).

Was found in the 4th group (which experimental diabetic retinopathy with correction of hyperglycemia, administration of aflibercept and L-arginine solution) at the 1st stage, an increase in the level of the vascular growth factor on 36.5 % (p<0.001), and compared to the 2nd in the 3rd groups, its values are less elevated on 18.6 % (p<0.001) and on 8 %, respectively. Was established at the second stage, an increase in the indicator on 24.7 % (p<0.001) compared to the intact group. It is noteworthy that the increase in the marker is less pronounced relative to the 2nd group on 43.6 % (p<0.001), relative to the 3rd group on 25.2 % (p<0.001), and in comparison, with the data of the group of the previous stage – on 3.8 % (p<0.001). At the third stage, the effect of this method of correction is even more pronounced: the level of the proliferation marker compared to the intact group is higher on 14.2 % (p<0.001), compared to group No. 2 it is lower on 53.4 % (p<0.001), with the 3rd, it is less pronounced on 36.3 % (p<0.001). Also, its value is smaller compared to the results of its group in the 1st stage – on 12.6 % (p<0.001), in the 2nd stage – on 9.2 % (p<0.001).

In the fifth group (which the pathological condition was corrected with hyperglycemic drug, the administration of aflibercept and bromfenac), the following data were obtained. At the 1st stage, the level of VEGF is higher on 42.8 % (p<0.001) compared to the intact group and on 4.6 % (p<0.01) relative to group 4. In comparison with group 2, its increase is less

pronounced on 14.9 % (p<0.001), and in comparison, with group 3 – on 3.9 % (p<0.05). At the second stage, the level of VEGF is higher on 33.5 % (p<0.001) compared to the 1st group, but the increase is less pronounced compared to the 2nd group – on 39.6 % (p<0.001), compared to the 3rd – on 19.9 % (p<0.001), compared to the group of the 1st stage – on 1.5 %. And compared to group 4, the proliferation marker increased on 7 % (p<0.001). At the third stage, VEGF increased on 28.9 % (p<0.001) compared to group 1, and on 12.9 % (p<0.001) compared to group 4. In relation to all subsequent comparison groups, its increase is less pronounced - compared to group No. 2 – on 47.4 % (p<0.001), group No. 3 – on 28 % (p<0.001), its own group of the 1st stage – on 5.7 % (p<0.01), and by its 2nd stage group – on 4.3 % (p<0.05).

It was established in the sixth group (which experimental animals were corrected for hyperglycemia, aflibercept, L-carnitine, and bromfenac) at the first stage, the level of vascular growth factor is 32.4 % (p<0.001), and relative to all other groups, its rise is less pronounced: on 21 % (p<0.001) compared to group 2, on 10.83 % (p<0.001) compared to group 3, on 3 % (p<0.05) compared to the 4th group, and on 7 % relative to the 5th (p<0.001). The established positive trend is followed also at the 2nd and 3rd stages of the experiment, which indicates the effectiveness of this method of correction.

The most pronounced positive effect is observed when using a hypoglycemic drug in combination with the administration of aflibercept, L-arginine solution and citicoline (in group No. 7). At the first stage, the level of VEGF is higher on 15.9 % (p<0.001), relative to all other groups of the experiment, its level is lower: compared to the 2nd group – on 30.9 % (p<0.001), compared to the 3rd – on 21.9 % (p<0.001), relative to group 4 it is lower on 15 % (p<0.001), relative to group 5 – on 18.8 % (p<0.001), and compared to the 6th group – on 12.4 % (p<0.001). At the second stage, the positive effect of corrective agents on the level of the proliferation marker is preserved - compared to the intact group, it is statistically insignificant and is 3.9 % higher. Relative to the group without correction, its value is lower on 52.9 % (p<0.001), and its value is also lower compared to all previous groups in which the pathological condition was corrected - relative to the 3rd group – on 37.6 % (p< 0.001), relative to the 4th - on 16.6 % (p<0.001), compared to the 5th – on 22.1 % (p<0.001), and relative to the 6th – on 13.7 % (p <0.001). Compared to the previous stage of its group, the rise is less pronounced on 5.6 % (p<0.01). At the third stage, the level of the marker is as close as possible to the values of the intact group - the difference is 0.9 % and is statistically insignificant. Compared with the data of other groups, the level of this method of correction is closer to the normal values – on 58.8 % (p<0.001) relative to the 2nd group, on 43.7 %

(p<0.001) relative to the 3rd, on 11, 7 % (p<0.001) relative to the 4th, on 21.8 % (p<0.001) compared to group 5, and on 13.7 % (p<0.001). Compared to the data of the same group on the 180th day, the result is better on 9.2 % (p<0.001) compared to the 1st stage, and on 3.8 % compared to the 2nd.

The obtained data confirm the development of diabetic retinopathy, a particularly marked increase in the marker was detected on the 180th day of the development of the pathological process (p<0.001). Aflibercept has a positive effect on the normalization of this condition, but a more pronounced effect is observed in combination with long-term administration of L-arginine. The relationship between the normalization of the vascular growth factor and the correction of hypoxia was also revealed.

Conclusions:

1.   The results of the analysis of the vascular growth factor in the 2nd group confirm the development of diabetic retinopathy, a particularly pronounced increase in the marker was detected on the 180th day of the development of the pathological process.

2.   It was established analyzing the data of group No. 3 that the correction of the pathological condition with hypoglycemic agents has partial positive effect but requires the involvement of additional means of correction in addition to the normalization of the level of glycemia.

3.   The results of the 4th group indicate that the involvement of a nitric oxide donor and aflibercept in the correction of diabetic retinopathy has positive effect on the reduction of the level of vascular growth factor, more pronounced compared to the 3rd group, but it does not reach normative values.

4.   It is observed that the correction of the simulated pathological condition by reducing hyperglycemia, administration of aflibercept and bromfenac (group No. 5) gives positive results, but less pronounced, compared to the data of the 4th group.

5.   It was found that in rats in which diabetic retinopathy was modeled with subsequent correction of hyperglycemia, administration of aflibercept, L-carnitine and bromfenac (group No. 6) at the first stage, there is a pronounced effectiveness of the proposed method of correction in comparison with the previously considered methods, the established positive trend is being followed also at the 2nd and 3rd stages of the experiment, which indicates the effectiveness of this correction method.

6. The most pronounced positive effect of normalizing the level of vascular growth factor is observed when using a hypoglycemic drug in combination with the administration of aflibercept, a solution of L-arginine and citicoline (in group No. 7).

References:

1. Vorob'eva I. V., Repkina M. Ju. Prakticheskie rekomendacii o nabljudenii bol'nyh s diabeticheskoj retinopatiej // RMZh. Jendokrinologija. —2009. — T. 17, № 24. — S. 1591-1595.

2. Ametov A. S. Saharnyj diabet i serdechno-sosudistye zabolevanija [Jelektronnyj resurs] / A.S. Ametov, I.O. Kurochkin, A.A. Zubkov // RMZh. – 2014. – № 13. – S. 954–959. – Rezhim dostupa: http://www.rmj.ru/articles_9505.htm

3. Abbate M, Cravedi P, Iliev I, et al. Prevention and treatment of diabetic retinopathy: evidence from clinical trials and perspectives // Curr Diabetes Rev. — 2011. — Vol. 7. — P. 190-200.

4. Vorob'eva I.V., Gigineishvili D.N. Rol' disfunkcii jendotelija v patogeneze diabeticheskoj retinopatii u bol'nyh saharnym diabetom 2 tipa. Obzor. Oftal'mologija. 2012;9(3):9-13.

5. Borzilova Ju.A.Boldyreva L.A.Shlyk I.V.Shurygina I.P.Gacu M.V. Uroven' VEGF-A v sleznoj zhidkosti pri diabeticheskoj retinopatii. Kubanskij nauchnyj medicinskij vestnik. 2015. № 6 (155). S.16-18.

6. Vorob'eva I.V., Merkushenkova D.A. Diabeticheskaja retinopatija u bol'nyh saharnym diabetom vtorogo tipa. Jepidemiologija, sovremennyj vzgljad na patogenez. Obzor. Oftal'mologija. 2012;9(4):18-21

7. Distler J. W., Hirth A., Kurowska-Stolarska M. Angiogenic and angiostatic factors in the molecular control of angiogenesis // Q J Nucl Med. — 2003. — Vol. 47. — P. 149-161.

8. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress // Endocr. Rev. — 2004. — Vol. 25. — P. 581-611.

9. Rangasamy S., McGuire P. G., Das A. Diabetic retinopathy and inflammation: novel therapeutic targets // Middle East Afr J Ophthalmol. — 2012. — Vol. 19. — P.52-59.

10. Konenkov V.I., Klimontov V.V., Chernyh V.V., Tjan N.V. Angiogenez pri proliferativnoj diabeticheskoj retinopatii: perspektivy anti-VEGF-terapii (obzor literatury). Oftal'mohirurgija.— 2013.— № 4.— S. 111-115.

11. Poveshhenko A.F., Konenkov V.I. Mehanizmy i faktory angiogeneza // Uspehi fiziol. nauk.— 2010.— T. 41, № 2.— P. 68-89.

12. Tongers J., Roncalli J.G., Losordo D.W. Role of endothelial progenitor cells during ischemia-induced vasculogenesis and collateral formation // Microvasc. Res.— 2010.— Vol. 79, № 3.— P. 200-206.

13. Willard A.L., Herman I.M. Vascular complications and diabetes: current therapies and future challenges // J. Ophthalmol. Pablished online – 2012.— Doc.10.1155/2012/ 209538.

14. Poltorak V.V., Bloh K.O., Malashenko A.M. Jeksperimental'noe modelirovanie saharnogo diabeta dlja izuchenija specificheskogo jeffekta novyh antidiabeticheskih veshhestv (Metod. rekomend.). Har'kov, 1991. 20 s.

15. Pasechnikova N. V. Zashhitnoe dejstvie kvercetina i lipoata na funkcional'nye gruppy belkov setchatki pri modelirovanii diabeta / N.V. Pasechnikova, O.A. Moroz // Oftal'mologichnij zhurnal. – 2015. – № 3. – S. 76-81

16. Jeksperimental'naja model' saharnogo diabeta 2-go tipa u krys, vyzvannaja dietoj s vysokim soderzhaniem zhirov i streptozotocinom v nizkoj doze / O.A. Kajdash, V.V. Ivanov, A.I. Vengerovskij, E.E. Bujko, I.A. Shhepetkin // Bjulleten' sibirskoj mediciny. – 2020. – №19(2). – S.41-47.

17. Rol' metformina v profilaktike diabeticheskoj nefropatii pri jeksperimental'nom saharnom diabete 2 tipa / V.K. Bajrasheva, A.Ju. Babenko, Ju.V. Dmitriev, A.A.Bajramov, S.G. Chefu, I.S. Shatalov, A.N. Aref'eva, I.Ju. Pchelin, N.V. Hudjakova, P.G. Aliev, E.N. Grineva // Regionarnoe krovoobrashhenie i mikrocirkuljacija. – 2016. – №15(3). – S.70-80.

18. Pokrovskij M.V. Jendotelioprotektornye jeffekty L-arginina pri modelirovanii deficita okisi azota / M.V. Pokrovskij, T.G. Pokrovskaja V.I. Korchakov // Jeksperimental'naja i klinicheskaja farmakologija. – 2008. – № 71 (2). – S. 29–31.

19. Efficacy of Subconjunctival Aflibercept Versus Bevacizumab for Prevention of Corneal Neovascularization in a Rat Model / Orly Gal-Or 1, Eitan Livny, Ruti Sella, Yael Nisgav, Dov Weinberger, Tami Livnat, Irit Bahar // Cornea. – 2016. – Vol. 3. – Issue 7. – R. 991-996.

20. Savytskyi. I. V.; Levytskyi, I. M.; Levytska, G. V.; Miastkivska, I. V.; Kuzmenko, I. A. Dynamics of vasoconstriction and vasodilation potential on the background of experimental rhegmatogenous retinal detachment and its correction. Journal of Education, Health and Sport 2016, 6 (2), p. 367-375.

21. Pavlova O. N. Issledovanie dinamiki aktivnosti katalazy v syvorotke krovi krys pri mehanicheskom vozdejstvii na gematooftal'micheskij bar'er / O. N. Pavlova, O. N. Gulenko, R. G. Karimova i dr. // Mezhdunarodnyj nauchno-issledovatel'skij zhurnal. — 2020. — № 5 (95) Chast' 1. – S.153—158.

22. Bykov I.L. Vlijanie L-karnitina na metabolicheskie narushenija pri jeksperimental'noj nedostatochnosti acil-KoA degidrogenaz/ I.L. Bykov // Jeksperimental'naja i klinicheskaja farmakologija. – 2004. – Tom 67 – № 6. S.48-52.

23. Dzugkoev S. G. Vlijanie kojenzima Q 10, afobazola i L-karnitina na jendotelial'nuju funkciju u krys s jeksperimental'nym saharnym diabetom / S.G. Dzugkoev, F.S. Dzugkoeva, N.V. Gumanova, V.A. Metel'skaja // Kubanskij nauchnyj medicinskij vestnik. – 2012. – №3 (132). – S.48-51.

24. Tkachenko A. S. Uroven' VEGF i FNO-α pri hronicheskom karragenaninducirovannom gastrojenterokolite. Visnik problem biologiï i medicini – 2013 – Vip. 3, Tom 2 (103). – S.231-234

25. Sirman Ya. V., Preys N. I., Savitsky I. V., Badiuk N. S., Blavatska O. M, Hrytsan I. I., Tsypoviaz S. V. Dynamics of vasoconstructor-vasodilation potential on the background of the development of experimental diabetic retinopathy / PharmacologyOnLine; Archives - 2021 - vol.1 – 90-95.