

MARKERS OF ENDOGENOUS INTOXICATION IN RATS WITH DIABETES MELLITUS COMBINED WITH CARRAGEENAN-INDUCED ENTEROCOLITIS

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Abstract

Background and aims: Diabetes mellitus (DM) is a significant public health issue, being one of the major contributors to morbidity and mortality in the modern societies. Chronic hyperglycemia produces significant physiological, biochemical, and histological changes in the affected organisms. This study aims to evaluate the markers of endogenous intoxication in rats with diabetes mellitus combined with carrageenan-induced enterocolitis. **Materials and methods:** Diabetes mellitus was induced by a single intraperitoneal administration of streptozotocin (Sigma Aldrich, USA, at a dose of 60 mg/kg body weight). Carrageenan-induced enterocolitis was modeled by giving the animals free access to 1.0 % solution of carrageenan in drinking water for 1 month. The syndrome of endogenous intoxication was assessed by quantification of low and medium molecular weight substances in blood plasma, red blood cell suspension and urine using extraction-spectrophotometric method. **Results:** The increase in endogenous intoxication in streptozotocin-induced diabetes combined with chronic enterocolitis can mainly be attributed to the catabolic pool of blood plasma substances of low and medium molecular weight. The index of distribution of low- and medium-molecular-weight substances between blood plasma proteins and glycocalyx of erythrocytes in the experimental groups increased simultaneously with the quantities of investigated fractions in the erythrocyte suspension measured at the wavelengths of 242, 254 and 280 nm. **Conclusions:** We observed upsurge of endogenous intoxication markers in the rats with diabetes mellitus. Endotoxycosis became even more evident in the rats with diabetes mellitus combined with carrageenan-induced enterocolitis.

key words: endogenous intoxication; diabetes; enterocolitis.

Background and aims

Diabetes mellitus (DM) is a significant public health issue as one of the major contributors to morbidity and mortality in the modern societies. It is a complex, chronic illness

requiring continuous medical care including multifactorial risk-reduction strategies in addition to glycemic control [1,2]. Disease burden attributed to diabetes is high and rising in every country, fuelled by the global rise in the prevalence of obesity and unhealthy lifestyles. A

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2014 study shows a global prevalence of 422 million people with diabetes, expecting to rise to 592 million by 2035 [3,4]. By 2040, the total number of diabetics worldwide is projected to increase to 642 million resulting in an increased economic burden [5]. The prevalence of diabetes varies in different populations depending on the factors such as genetic and cultural background, ranging, for instance, from 10 % in the Japanese to 40 % in Pima Indians [6]. In the Europe Region, the number of people with diabetes in 2013 was estimated at 56 million with an overall estimated prevalence of 8.5% [7].

Chronic hyperglycemia causes significant physiological, biochemical, and histological changes in the affected organisms [8]. Glucose-, lipid- and insulin- toxicity produced by carbohydrate and lipid metabolism disorders associated with diabetes is the main factor contributing to generation of a large number of reactive oxygen species (ROS). ROS increase lipid peroxidation and oxidative modification of proteins against the backdrop of antioxidant defense system exhaustion, increased glycosylation of structural receptor proteins, anaerobic glycolysis, and accumulation of abnormal products of lipid metabolism. At the same time, detoxification function of the liver suffers, possibly resulting in diabetic hepatopathy, a disorder that occurs in 64-88 % of the patients with DM. These destructive processes, which are associated with a violation of the structure and function of membranes, cause metabolic changes that form the syndrome of endogenous intoxication [9].

An important marker of endotoxemia are the levels of middle molecular weight substances (MMWS), a heterogeneous group of substances of various structures with molecular weight ranging from 300 to 5000 Da [10-12]. MMWS include substances of low and medium molecular weight, containing catabolic and

anabolic pools and oligopeptides, with a molecular weight of less than 10 kDa. Substances of low and medium molecular weight include creatinine, urea, oligosaccharides, lactic acid, bilirubin, amino acids, cholesterol, lipid peroxidation products and other compounds. They are distributed in the blood among plasma proteins, micelles of different classes of lipoproteins and erythrocyte glycocalyx, capable of transporting these substances. Oligopeptides consist of regulatory peptides (neurotensins, somatostatin, vasoactive intestinal peptide, enkephalins and other biologically active substances) and on-regulatory peptides (products of proteolytic degradation of plasma and tissue proteins that enter the blood as a result of autolysis, ischemia, organ hypoxia, and proteolytic processes) [13].

According to research by Malakhova [14], the catabolic pool of the MMWS which can be detected at the wavelength range of 242 to 258 nm consists of protein catabolism products and low molecular weight metabolites such as urea, creatinine, uric acid, purine metabolism products, as well as nucleotides and their derivatives, nucleoprotein metabolites. A significant increase in the number of catabolic products is one of the stages in the development of endogenous intoxication syndrome. Anabolic pool of the MMWS can be detected at the wavelength range of 258 - 298 nm. This group includes mainly fragments of protein molecules containing aromatic amino acids, metabolites of the urea cycle, purine and pyrimidine, and their derivatives.

This study tracked changes to the markers of endogenous intoxication in rats with diabetes mellitus combined with carrageenan-induced enterocolitis.

Material and methods

The experimental animals, white nonlinear mature male rats (n=48) were kept on a standard diet at the animal facility of I. Horbachevsky Ternopil State Medical University. Animal treatment and all experimental procedures were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [15].

The experimental design included four groups: control group (n=12); animals with diabetes mellitus (n=12); animals with carrageenan-induced enterocolitis (n=12); and animals with diabetes mellitus and carrageenan-induced enterocolitis (n=12). Diabetes mellitus was induced by a single intraperitoneal administration of streptozotocin (Sigma Aldrich, USA, at a dose of 60 mg/kg body weight) [16]. Immediately prior to the administration, streptozotocin was dissolved in 0.1 M citrate buffer (pH 4.5). The control group received a corresponding amount of citrate buffer. Animals with glucose level of at least 10.8 mmol/L in 2 weeks after streptozotocin administration were included in the DM experimental groups.

Carrageenan-induced enterocolitis (CEC) was modeled by giving the animals free access to 1.0% solution of carrageenan in drinking water for 1 month [17]. We used blood serum and colon homogenate of the rats to determine the markers. Concentration of glucose in blood serum was determined using a glucometer (MediSense UK Ltd., Great Britain).

Syndrome of endogenous intoxication was evaluated using measurements of low and medium molecular weight substances contents in blood plasma, red blood cell suspension and urine quantified by extraction-spectrophotometric method [18]. High molecular weight substances of blood plasma, erythrocytes, and urine were precipitated in 15% solution of

trichloroacetic acid. Trichloroacetic extracts of blood plasma and red blood cells were measured by spectrophotometer SF-200 at the wavelengths of 242, 254 and 282 nm, trichloroacetic extracts of urine - at wavelengths of 236, 254 and 282 nm. The obtained data are expressed in standard units of optical density (U). Using the data, the following indices were calculated to evaluate the intensity of endogenous intoxication [12]:

1. Ct, total contents of low and medium molecular weight substances in plasma:

$$Ct = (E242 + E254 + E282) \times 40;$$

2. Cc, the value of catabolic pool of low and medium molecular weight substances in plasma:

$$Cc = (E242 + E254) \times 12;$$

3. Pc%, catabolic pool of plasma:

$$Pc\% = Cc / Ct \times 100\%;$$

4. ICP, intensity of catabolic processes in plasma:

$$ICP = (E242 + E254) / (E254 + E282);$$

5. K1, distribution rate of low and medium molecular weight substances between blood plasma proteins, and erythrocyte glycocalyx:

$$K1 = (E242 + E254 + E282) \text{ of plasma} / (E242 + E254 + E282) \text{ of erythrocytes};$$

6. K2, elimination process condition, indicating the ability of kidneys to excrete endotoxemia products:

$$K2 = (E236 + E254 + E282) \text{ of urine} / ((E242 + E254 + E282) \text{ of plasma} + (E242 + E254 + E282) \text{ of erythrocytes}).$$

Nucleic acids levels were determined out using the A.S. Spirins method: extraction of nucleic acids from the blood plasma by the 10% solution of HClO₄. Their contents were measured by spectrophotometer SF-200 at the

wavelengths of 270 and 290 nm [19]. The results were expressed in conventional units (CU).

The data were processed using the software package Statistica 6.1 for Windows. Intergroup comparisons were performed using Mann–Whitney U test. The median (Me) and interquartile range (IQR (Q25-Q75)) were calculated. Differences with p-value of ≤ 0.05 were accepted as statistically significant.

Results

In the case of experimental DM, the levels of low and medium molecular weight substances in

plasma and red blood cells have increased (Table 1). Thus, the fraction of these substances with a maximum absorption of 242 nm in blood plasma significantly increased: in animals with DM and in animals with CEC by 2.0 times and in animals with DM and CEC by 2,6 times ($p < 0,001$). The index E_{242} in red blood cell suspension significantly increased: in animals with DM by 1.5 times, in animals with CEC and in animals with DM and CEC by 1.8 times.

Table 1. The indices of endogenous intoxication in blood of the control and experimental groups, Me (Q25-Q75).

Index	Control	DM	CEC	DM+CEC
	Blood plasma			
E_{242}	0.09 (0.06; 0.10)	0.18* (0.14; 0.17)	0.18* (0.14; 0.21)	0.23*^# (0.21; 0.25)
E_{254}	0.27 (0.22;0.31)	0.54 * (0.51; 0.56)	0.57 * (0.53; 0.62)	0.70 *^# (0.67;0.75)
E_{280}	0.23 (0.19; 0.27)	0.32 * (0.30; 0.35)	0.37 * (0.35; 0.39)	0.44 *^# (0.39; 0.46)
	Red blood cell suspension			
E_{242}	0.17 (0.18;0.18)	0.25 * (0.23;0.27)	0.30* (0.29;0.31)	0.30*^ (0.28;0.33)
E_{254}	0.45 (0.41;0.49)	0.56* (0.51;0.60)	0.59* (0.56;0.61)	0.70*^# (0.66;0.77)
E_{280}	0.19 (0.12;0.24)	0.29* (0.26;0.32)	0.34* (0.32;0.35)	0.36*^# (0.35;0.39)
Notes: * – the difference between the control and the experimental group is statistically significant ($p \leq 0.05-0.001$); ^ – the reliability coefficient between groups 2 and 4; # – the reliability coefficient between groups 3 and 4.				

In blood plasma fractions of substances with a maximum absorption of 254 nm significantly increased: in animals with DM by 2.0 times, in animals with CEC by 2.1 times, and in animals

with DM and CEC by 2.6 times ($p < 0,001$). In red blood cell suspension, the acid-soluble fraction E_{254} significantly increased: in animals with DM

by 1.2 times, in animals with CEC by 1.3 times, and in animals with DM and CEC by 1.6 times.

The increase of the acid-soluble fraction of substances at the wavelength of 280 nm in plasma and erythrocyte suspension in all experimental groups was due to the accumulation of biologically active substances with molecular weight ranging from 2000 to 5000 Da. Thus, in blood plasma, the fraction of these substances with a maximum absorption of 280 nm significantly increased: in animals with DM by 1.4 times, in animals with CEC by 1.6 times and in animals with DM and CEC by 1.9 times ($p < 0.01$). In red blood cell suspension, the index

E_{280} significantly increased: in animals with DM by 1.5 times, in animals with CEC by 1.8 times and in animals with both DM and CEC by 1.9 times. This wavelength detects substances that contain aromatic amino acids, in particular tyrosine, tryptophan, and phenylalanine.

Analysis of the contents of low and medium molecular weight substances in urine showed that the fractions of substances at maximum absorption of 236, 254 and 280 nm in of the animals with DM and DM coupled with CEC did not differ from those in the control group. In animals with CEC E_{236} significantly increased by 16.7 % and E_{254} by 13.6 % ($p < 0.05$) (Table 2).

Table 2. The indices of endogenous intoxication in urine of the control and experimental groups, Me (Q25-Q75).

Index	Control	DM	CEC	DM+CEC
E_{236}	0.48 (0.45;0.52)	0.49 (0.46;0.53)	0.56* (0.53;0.58)	0.48 (0.47;0.51)
E_{254}	0.44 (0.41;0.48)	0.45 (0.42;0.47)	0.50* (0.46;0.54)	0.43 (0.40;0.44)
E_{280}	0.40 (0.32;0.48)	0.42 (0.36;0.45)	0.44 (0.41;0.45)	0.38 (0.31;0.45)
Notes: * – the difference between the control and the experimental group is statistically significant ($p \leq 0.05-0.001$); ^ – the reliability coefficient between groups 2 and 4; # – the reliability coefficient between groups 3 and 4.				

Analysis of the calculated coefficients indicates that the total pool of middle molecular weight substances in blood plasma was significantly higher in all experimental groups compared to the control. It should be noted that in rats with DM combined with CEC, Ct coefficient was significantly higher than this index in animals with DM (by 31.8 %) and in animals with CEC (by 22.5 %) (Table 3).

The increase in endogenous intoxication in the Group 4 animals can mainly be attributed to the catabolic pool of blood plasma substances of low and medium molecular weight. The index of distribution of the substances of low and medium molecular weight between the proteins of blood plasma and glycocalyx of erythrocytes in the

experimental groups increased due to the high values at wavelengths of 242, 254 and 280 nm in the investigated fractions of erythrocyte suspension. These data indicate a decrease in the adsorption capacity of erythrocytes, especially in the group of animals with DM and CEC (Table 3). In all experimental groups, the ability of the kidneys to eliminate toxic products is significantly reduced. Thus, the index of the elimination of toxic products significantly decreased: in animals with DM by 1.5 times, in animals with CEC and in animals with DM and CEC by 1.8 times ($p < 0.001$). Our results indicate that in the case of DM combined with CEC increase in the levels of low and medium molecular weight substances occurs due to their

excessive formation in the tissues of the body and disruption of the elimination processes.

The metabolic outcome of endotoxycosis is the increased cellular degradation and other pathological processes. To confirm the hypothesis of cell death, we determined the level of nucleic acids in the blood of rats. In all experimental groups, the nucleic acids levels

were significantly higher than in the rats of control group: in animals with DM by 65.7 %, in animals with CEC by 39.5 % and in animals with both DM and CEC by 76.2 % (Figure 1). We believe that in our experiment, the main source of nucleic acids is the apoptosis of nucleated cells, but this hypothesis requires further studies.

Table 3. Markers of the intensity of endogenous intoxication in rats with carrageenan-induced enterocolitis combined with diabetes mellitus (M±m).

Index	Control	DM	CEC	DM+CEC
Ct	23.13±1.13	41.27±0.94*	44.43±1.17*	54.43±1.03* p _{1,2} <0.001
Cc	4.24±0.27	8.59±0.26*	8.92±0.37*	11.11±0.25* p _{1,2} <0.001
Pc%	18.29±0.70	20.81±0.40*	20.04±0.37*	20.41±0.18*
ICP	0.72±0.03	0.85±0.03*	0.80±0.02*	0.82±0.01*
K ₁	0.72±0.02	0.95±0.02*	0.91±0.03*	1.01±0.03* p ₂ =0.04
K ₂	0.97±0.05	0.64±0.02*	0.55±0.01*	0.55±0.02* p ₁ =0.0001

Notes: * – the difference between the control and the experimental group is statistically significant (p≤0.05-0.001); ^ – the reliability coefficient between groups 2 and 4; # – the reliability coefficient between groups 3 and 4.

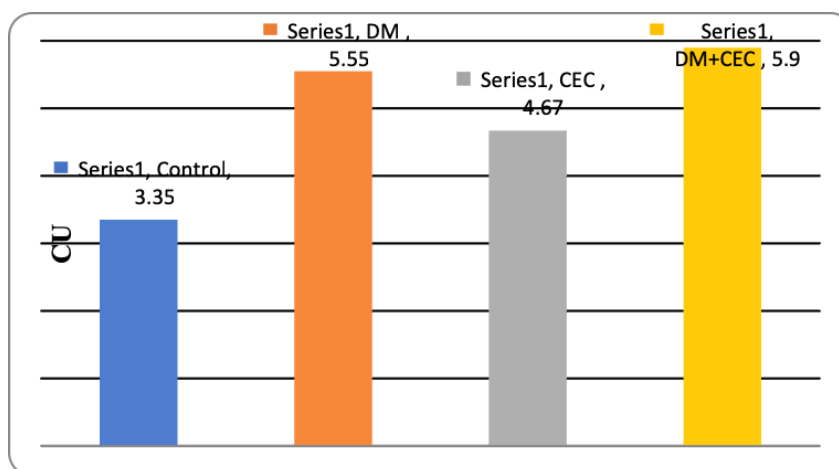


Figure 1. The levels of nucleic acids in the blood of rats with carrageenan-induced enterocolitis combined with diabetes mellitus.

Discussion

Endogenous intoxication syndrome is characterized by metabolic, morphological and functional disorders of various organs and systems and occurs in response to various factors of the external and internal environments, as a result of toxic substances accumulation in tissues and biological fluids: an excess of the products of normal and impaired metabolism or cellular response [12]. Today, endogenous intoxication syndrome is viewed as a process that contributes to the systemic inflammatory response syndrome [20]. Overstrain of adaptation mechanisms, breakdown of compensation, imbalance of reactions at the biomolecular level are understood to lead to structural and metabolic changes that cause development of homeostasis disorders. In this environment, many substances can acquire the properties of endotoxins, not being such in the normal physiological conditions. This concept is important since metabolic disorders do not manifest clear clinical picture in the early stages of their development. Syndromic diagnosis of impaired metabolism, as a rule, lags behind the events of developing pathological processes at the cellular and biochemical levels [21].

A significant feature of MMWS is their high biological activity. They have neurotoxic activity, inhibit protein synthesis, promote hemolysis of erythrocytes, inhibit erythropoiesis and enzymatic activity, and cause a state of secondary immunosuppression [10]. MMWS can also disrupt the processes of humoral regulation by blocking cell receptors, binding to the active centers of the albumin molecule, and competing with regulatory peptides [22]. The accumulation of MMWS and their disrupted distribution between plasma and erythrocytes, as well as the interruption of their excretion by the kidneys,

caused by various etiological factors, lead to the development of endogenous intoxication [13].

Consequently, in streptozotocin-induced diabetes combined with chronic enterocolitis, endogenous intoxication develops in the rats, characterized by a statistically significant increase in the total pool of substances of low and middle molecular weight in the plasma (by 2.4 times). The increase in the levels of molecular fractions detected at the wavelengths of 242, 254 and 280 nm is due to their excessive formation in the tissues of the body, mainly in the catabolic pool, coupled with a disruption of the processes of their elimination.

A metabolic outcome of these processes in rats with streptozotocin-induced diabetes combined with chronic enterocolitis is the increased cell death, which can be confirmed by 76.2 % increase in the nucleic acids levels in the blood of the animals ($p < 0,001$).

According to Payenok [23], the activation of lipid peroxidation processes is an important pathophysiological mechanism for the development of endogenous intoxication. Excessive lipoperoxidation is accompanied by the accumulation of peroxide oxidation products and depletion of antioxidant system reserves, which causes hyperenzymemia and accumulation of toxic substances [24,25].

Our previous studies [26] showed that the number of ROS in rats with DM increased by 3.0 times, in rats with CEC by 2.0 times, and in rats with combined pathology 3.4 times compared to the control values. In case of diabetes mellitus, the production of ROS increases, causing the development of oxidative stress. This can be due to auto-oxidation of glucose, enhanced glycosylation of cellular proteins, activation of the polyol pathway, and increased formation of superoxide radical in the respiratory chain of mitochondria [27]. Moreover, in diabetes mellitus, advanced glycation end products have

damaging effect on cell and tissue DNA, accumulating in the ganglia, villi and enterocyte membranes of the intestine [28].

Conclusions

We observed increase in the indices of endogenous intoxication in rats with diabetes

mellitus. A pronounced intensification of endotoxycosis was observed in rats with diabetes mellitus combined with carrageenan-induced enterocolitis.

REFERENCES

1. **Krynytska I, Marushchak M.** The indices of nitric oxide system in rats with carrageenan-induced enterocolitis combined with diabetes mellitus. *Rom J Diabetes Nutr Metab Dis* 25(3): 283-288, 2018.
2. **Grant RW, Kirkman MS.** Trends in the evidence level for the American Diabetes Association's "Standards of Medical Care in Diabetes" from 2005 to 2014. *Diabetes Care* 38: 6-8, 2015.
3. **Forouhi NG, Wareham NJ.** Epidemiology of diabetes. *Medicine (Abingdon)* 42(12): 698-702, 2014.
4. **World Health Organization.** Global report on diabetes, 2016. Accessed at: http://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf;jsessionid=BD444CE436D08DC3AAC6E367BC50C783?sequence=1
5. **Upadhyay J, Polyzos SA, Perakakis N et al.** Pharmacotherapy of type 2 diabetes: An update. *Metabolism* 78: 13-42, 2018.
6. **Riyaz MSU, Rather MK, Koul PA.** Diabetes in Immigrant Tibetan Muslims in Kashmir. *North India J Immigrant Minority Health* 5(1): 18-30, 2017.
7. **Tamayoa T, Rosenbauera J, Wildb SH et al.** Diabetes in Europe: An update. *Diabetes Res Clin Pract* 103(2): 206-217, 2014.
8. **Guzyk MM, Dyakun KO, Yanytska LV et al.** Inhibitors of Poly (ADP-Ribose) Polymerase-1 as Agents Providing Correction of Brain Dysfunctions Induced by Experimental Diabetes. *Neurophysiology* 49(3): 183-193, 2017.
9. **Botsyrko VI, Hryb VA, Gerasymchuk RD, Erstenyuk AM.** Endogenous intoxication and oxidative stress in patients with type 2 diabetes mellitus, complicated with polyneuropathy. *Архів клінічної медицини* 1: 22-25, 2010.
10. **Obukhova LM, Andriyanova NA.** The determination of the low and medium molecular weight substances in the blood serum as the additional diagnostic criterion in the cases of lethal poisoning with narcotic drugs. *Forensic medical expertise* 6: 37-39, 2014.
11. **Krynytska I, Marushchak M, Svan O, Akimova V, Mazur L, Habor H.** The indices of endogenous intoxication in rats with carrageenan solution consumption. *Georgian Med News* 279: 196-200, 2018.
12. **Koryakina YV, Belova SV.** Molecules of medium mass as an integral measure of metabolic disorders (literature review). *Clin Lab Diagn* 2: 3-8, 2004.
13. **Rubtsov GK.** Parameters of oxidative modification of proteins and lipids and middle mass molecules in the methodology for the evaluation of endotoxycosis in cholecystitis: monogr. *Grodno*:174, 2016.
14. **Malakhova MY.** Endogenous intoxication as reflection of compensatory reconstruction of metabolic processes in organism. *Efferent Therapy* 4: 3-14, 2000.
15. **Council of Europe Strasbourg.** European convention for the protection of vertebrate animals used for experimental and other scientific purposes 123: 52, 1986.
16. **Ordodi VL, Paunescu V, Ionac M et al.** Indomethacin inhibits thymic involution in mice with streptozotocin-induced diabetes. *Artificial organs* 32(1): 66-70, 2008.
17. **Moyana TN, Lalonde JM.** Carrageenan-induced intestinal injury in the rat-a model for inflammatory bowel disease. *Ann Clin Lab Sci* 20(6): 420-426, 1990.
18. **Urakova TY, Lysenkova NS.** Endogenous intoxication and adaptive possibilities of obese patients. *International Journal of Fundamental Investigations* 5: 39-45, 2009.
19. **Spirin AS.** Molecular biology: structure and protein biosynthesis. *M: High School* 350, 1990.

20. **Bone RS.** Sepsis, sepsis syndrome and the systemic inflammatory response syndrome (SIRS). *JAMA* 2 (273): 155–156, 1995.
21. **Shmoilov DK, Karimov IZ, Odinets TM.** Pathogenic role of endogenous intoxication. *Laboratory Diagnostics* 2(60): 65-69, 2012.
22. **Sidelnikova VI, Chernitskiy AE, Retsky MI.** Endogenous intoxication and inflammation: reaction sequence and informativity of the markers (review). *Agricultural Biology* 50(2): 152-161, 2015.
23. **Pajenok OS, Kostiv MO.** Lipid peroxidation processes and endogenous intoxication level in pregnant women with thyropathies. *Exper and Clin Physiology and Biochemistry* 1: 97–101, 2012.
24. **Korda MM.** Status of the antioxidant, monooxygenase and humoral immune system of the body in d-galactosamine hepatitis. *Ukrain'skyi Biokhimichni Zhurnal* 68(1): 72-75, 1996.
25. **Marushchak M, Krynytska I, Petrenko N, Klishch I.** The determination of correlation linkages between level of reactive oxygen species, contents of neutrophiles and blood gas composition in experimental acute lung injury. *Georgian Med News* (253): 98-103, 2016.
26. **Marushchak M, Lisnianska N, Krynytska I, Chornomydz I.** The mechanisms of apoptosis initiation in rats with chronic enterocolitis combined with streptozotocin-induced diabetes. *Georgian Med News* 9(270): 125-130, 2017.
27. **van den Oever I, Raterman HG, Nurmohamed MT, Simsek S.** Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators Inflamm* 2010: 792393, 2010.
28. **Babu K, Babu S, Walker J, Walker AB, Pappachan JM.** Gastrointestinal complications of diabetes mellitus. *World J Diabetes* 4(3): 51–63, 2013.