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Abstract

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INTENSITY OF OXIDATIVE STRESS AS A UNIVERSAL MECHANISM OF TISSUE DAMAGE IN NONALCOHOLIC STEATOHEPATITIS AND DIABETIC KIDNEY DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS. QUERCETIN CORRECTION EFFECTIVENESS

The objective of the research was to determine the intensity of the effect of a complex of Metformin, Rosuvastatin, Essentiale Forte N and Quercetin on the state of oxidative-antioxidant homeostasis, as well as the intensity of hepatocyte apoptosis by cytokeratin-18 in the blood that are the factors in the progression of nonalcoholic steatohepatitis and diabetic kidney disease.

Material and methods. 75 patients with non-alcoholic steatohepatitis with type 2 diabetes mellitus and stage I–III diabetic kidney disease were studied over time. According to the prescribed treatment, the examined patients were divided into 2 groups: (group 1 – the controls: 37 patients) received a low-calorie diet with dietary restrictions No.9, essential phospholipids (Essentiale forte H 300 mg 2 caps. 3 times a day) for 30 days for the treatment of active non-alcoholic steatohepatitis, Metformin Hydrochloride (Metformin-Teva) 1000 mg per day, Rosuvastatin (Rosuvastatin-Teva 5 mg once daily) for 1 month for concomitant type 2 diabetes mellitus and hyperlipidemia. Group 2 consisted of patients (38 people) who in addition to similar dietary recommendations and therapy received additionally Quercetin and Povidone (Corvitin) 500 mg intravenously per 100 ml of isotonic chloride for 10 days.

Results. It was found that the comorbid course of nonalcoholic steatohepatitis and diabetic kidney disease in patients with type 2 diabetes mellitus is accompanied by a significant increase in the intensity of oxidative stress, accompanied by an increase in blood intermediate and final products of lipid peroxidation. The damaging effect of oxidative stress in patients with type 2 diabetes mellitus leads to the activation of apoptosis of hepatocytes with an increase in blood cytokeratin-18 (7.5 times, $p < 0.05$), the content of which correlates with the degree of oxidative stress, the intensity of liver damage and stage of diabetic kidney disease ($p < 0.05$). Oxidative stress increases the risk of endothelial damage by atherosclerotic process due to hyperproduction of homocysteine (3.9 times, $p < 0.05$), which contributes to the progression of diabetic kidney disease. The use of Quercetin in the complex therapy of non-alcoholic steatohepatitis and type 2 diabetes mellitus with diabetic kidney disease contributes to the reduction of oxidative stress,

increased activity of antioxidant defense factors (content of reduced glutathione in erythrocytes, reduction of cytokeratin-18 content by 1.7 times) and endothelial damage (reduction of homocysteine content in blood by 1.9 times) ($p < 0.05$).

Conclusions. The comorbid course of nonalcoholic steatohepatitis and diabetic kidney disease in patients with type 2 diabetes mellitus is accompanied by a significant increase in the intensity of oxidative stress, and in the content of intermediate and final products of lipid peroxidation and oxidative modification of proteins ($p < 0.05$).

Keywords: non-alcoholic steatohepatitis, type 2 diabetes mellitus, diabetic kidney disease, oxidative-antioxidant homeostasis, apoptosis, atherosclerosis, Quercetin.

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Резюме

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ІНТЕНСИВНІСТЬ ОКСИДАТИВНОГО СТРЕСУ ЯК УНІВЕРСАЛЬНОГО МЕХАНІЗМУ ПОШКОДЖЕННЯ ТКАНИН ПРИ НЕАЛКОГОЛЬНОМУ СТЕАТОГЕПАТИТІ ТА ДІАБЕТИЧНІЙ ХВОРОБІ НИРОК У ХВОРИХ НА ЦУКРОВИЙ ДІБЕТ ТИПУ 2. ЕФЕКТИВНІСТЬ КОРЕКЦІЇ КВЕРЦЕТИНОМ

Мета роботи: було з'ясування інтенсивності впливу комплексу засобів метформіну, розувастатіна, Ессенціале форте Н і кверцетину на стан оксидантно-антиоксидантного гомеостазу, а також на інтенсивність апоптозу гепатоцитів за змістом в крові цитокератину-18, які є факторами прогресування неалкогольного стеатогепатиту і діабетичної хвороби нирок.

Матеріали та методи. Проведені дослідження в динаміці лікування у 75 хворих неалкогольного стеатогепатиту з цукровим діабетом типу 2 і діабетичної хвороби нирок I–III стадії. Згідно призначеного лікування обстежені хворі були розділені на 2 групи: (1 група – контрольна: 37 пацієнтів) отримували гіпокалорійну дієту з урахуванням обмежень дієти № 9, есенціальні фосфоліпіди (Ессенціале форте Н 300 мг по 2 капс. 3 рази на день) 30 днів з метою лікування активного неалкогольного стеатогепатиту, з приводу коморбідного цукрового діабету типу 2 і гіперліпідемії призначали метформіну гідрохлорид (Метформин-Тева) 1000 мг на добу, розувастатин (Розувастатин-Тева 5 мг 1 раз на день) протягом 1 місяця. 2 групу склали пацієнти (38 осіб), які, крім аналогічних дієтичних рекомендацій щодо дієти та лікування, додатково отримували препарат кверцетину і повідону (Корвітин) по 500 мг в 100 мл ізотонічного розчину натрію хлориду) в протягом 10 днів.

Результати дослідження. Аналіз отриманих даних показав, що до лікування у хворих обох груп порівняння було встановлено значній мірі оксидативного стресу, який супроводжувався істотним накопиченням в крові проміжних і кінцевих продуктів перекисного окислення ліпідів і окиснювальної модифікації білків. Так, до лікування вміст малонового альдегіду в плазмі крові перевищував референтні значення в 2,1 рази ($p < 0,05$), вміст ізольованих подвійних зв'язків – в 1,9 рази ($p < 0,05$), вміст альдегід-динітро-

фенілгідразонів – в 2,3 рази ($p < 0,05$). У той же час, стан системи антиоксидантного захисту був істотно розбалансований. Так, вміст у крові відновленого глутатіону був нижче показника у практично здорових осіб в 1,7 рази ($p < 0,05$), активність глутатіонпероксидази – була знижена в 1,3 рази ($p < 0,05$), що пояснює високу інтенсивність оксидативного стресу у обстежених хворих. Зазначені продукти перекисного окислення ліпідів і окиснювальної модифікації білків на тлі істотного недостатності системи антиоксидантного захисту привели до активації процесів апоптозу гепатоцитів. Свідченням цього є істотне підвищення вмісту в крові цитокератину-18 у 7,5 рази ($p < 0,05$) в порівнянні з практично здоровими особами.

Висновки. Коморбідний перебіг неалкогольного стеатогепатиту і діабетичної хвороби нирок у хворих на цукровий діабет типу 2 супроводжується істотним зростанням інтенсивності оксидативного стресу, супроводжується зростанням вмісту в крові проміжних і кінцевих продуктів перекисного окислення ліпідів і окиснювальної модифікації білків в межах 1,9–2,3 рази ($p < 0,05$).

Ключові слова: неалкогольний стеатогепатит, цукровий діабет типу 2, діабетична хвороба нирок, оксидантно-антиоксидантний гомеостаз, апоптоз, атеросклероз, кверцетин.

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Introduction/Вступ

Every year in Ukraine and the world the incidence of non-alcoholic steatohepatitis (NASH) in patients with obesity and type 2 diabetes mellitus (DM2) is significantly increasing [1, 2]. These diseases have a large number of common etiopathogenetic links and mechanisms of mutual burden. The intensity of damage factors increases with the development of diabetic kidney disease (DKD) [1, 2, 3, 4].

Oxidative stress (OS) occupies a leading place in the mechanisms of progression of NASH and DKD in patients with diabetes mellitus [5, 1, 2, 3]. The increase in the intensity of OS under the influence of various inducers underlies the transformation of nonalcoholic steatosis of the liver into NASH, the development and progression of inflammatory-necrotic changes in the liver in NASH, as well as liver fibrosis [1]. Enhancement of lipid peroxidation (LPO) and oxidative modification of proteins (OMP) of organelle membranes is accompanied by swelling of mitochondria, increased permeability of lysosomal membranes, systemic disruption of cell membrane integrity [5, 2, 3]. LPO products stimulate collagen formation in the liver and kidneys [1], as well as cause the formation of Mallory cells, which involves the deposition of cross-linked cytokeratin monomers [6, 2]. Accumulation of endotoxins, intermediate and end products of LPO on the background of impaired carbohydrate and lipid metabolism contribute to the induction of cytochrome P450 (Cyp2E1), which is accompanied by the release of large amounts of free radicals of oxygen (FRO) and nitrogen [7]. Increased oxygen consumption by hepatocytes is also accompanied by the formation of FRO and increased processes of LPO and OMP [5, 8, 3]. In NASH, there is increased activity of cytochrome P450 in the liver, which is able to generate FRO in the process of detoxification of free fatty acids (FFA), aldehydes, ketones, N-nitrosamines, and other endotoxins and exotoxins [1]. Initiation of necrotic processes in liver tissue is also a consequence of FRO hyperproduction [2]. An important role in the attachment of the inflammatory component is

played by the processes of LPO of structural membrane lipids, which induce the processes of apoptosis and cytolysis of hepatocytes [5, 1]. Evidence of this is a significant increase in the expression on lymphocytes of the marker of apoptosis Fas Apo-1 (CD95) against the background of a significant increase in LPO processes [1]. This is facilitated by hyperproduction of proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, TGF-β1), which occurs in response to endotoxinemia [2]. The entry of lysosomal hydrolases and other components of the hepatocyte into the intercellular space and into the systemic circulation is a signal to the induction of a cascade of reactions in response to damage [6, 1]. A further scenario involves the activation of cell adhesion molecules (ICAM-1, ICAM-2), polymorphonuclear infiltration of liver tissue, microcirculation disorders, ie the progression of dystrophic and inflammatory-necrotic changes in liver tissue [2]. By a similar mechanism, OS affects the endothelium, causing the development of endothelial apoptosis, its accelerated exfoliation and endothelial dysfunction (ED). OS promotes the development of micro- and macroangiopathies in patients with diabetes mellitus 2 with the development of angiospasms and in general – disruption of microcirculation and oxygen supply to peripheral tissues, liver and kidney parenchyma, which causes progression of NASH and DKD [6, 5, 1, 2].

Oxidative stress is counteracted by antioxidant defense systems (AODS). The leading system of the natural detoxification system and AODS is the glutathione system. Performing the functions of the universal redox system, glutathione and a number of enzymes that serve it protect cell membranes from the effects of FRO, nitrogen (peroxynitrite), hydroperoxides, and binds hydrophilic products of microsomal oxidation (first phase of detoxification) and provides the second phase conjugation) with the excretion of non-toxic compounds from the body [1, 2]. Under conditions of comorbidity of pathological conditions, which are accompanied by a significant degree of OS, it is important to monitor the state of the AODS, because the glutathione link is constantly depleted and requires periodic replenishment. In our previous studies, it was proved that appropriate control over the content of reduced glutathione in erythrocytes makes it possible to adequately assess the body's need to restore and stimulate the antioxidant system (AOS) in general in order to counteract OS [5, 1, 2]. Thus, a large number of studies on this comorbidity indicates a significant relevance of this problem, and a number of unresolved issues concerning the study of the mechanisms of cell damage by induction of apoptosis, as well as control over the intensity of their progression against the background of oxidative-antioxidant imbalance and, most importantly, the development of treatment methods by monitoring the state of the AOS and reducing the intensity of damaging free radical effects.

In terms of counteracting free radical effects in clinical practice, Quercetin is a flavonoid of herbal origin, which inhibits the intensity of LPO and OMP membranes, stimulates the activity of catalase and superoxide dismutase (SOD) in cells [9, 6, 10, 11, 12]. Quercetin restores the ability of the endothelium to synthesize NO, which explains its cardioprotective effect in ischemic and reperfusion heart disease [9, 10, 13, 14, 15]. The drug has a powerful anti-inflammatory effect, inhibiting 5-lipoxygenase, cyclooxygenase, hyaluronidase, a number of proteases, calcium-dependent ATPase, synthesis of leukotrienes LTC4 and LTB4, has immunomodulatory properties, thus, reducing the area of necrotized myocardium and enhancing reparative processes [16, 17, 7, 18, 19]. There are a number of reports of hypolipidemic, choleretic, anticholestatic, hepatoprotective properties of Quercetin, established in obese people and in patients with NASH [9, 10, 8, 20, 3, 4, 21–24]. At the same time, the complex effect of Quercetin on the functional state of the LPO-AODS, the intensity of apoptosis and the factors that regulate them in patients with NASH and DKD on the background of diabetes mellitus 2 has been studied in limited patients or experimentally.

The objective of the study was to determine the effect of a complex of Metformin, Rosuvastatin, Essentiale Forte N and Quercetin on the state of oxidative-antioxidant homeostasis, as well as the intensity of apoptosis of hepatocytes by blood cytokeratin-18 content, which are factors in the progression of NASH and DKD.

Material and methods. 75 patients with non-alcoholic steatohepatitis with type 2 diabetes mellitus and stage I–III diabetic kidney disease were studied over time. According to the prescribed treatment, the examined patients were divided into 2 groups: (group 1 – the controls: 37 patients) received a low-calorie diet with dietary restrictions No.9, essential phospholipids (Essentiale Forte N (Sanofi–Avensis / Natterman and Cie mg GmbH) 2

caps. 3 times a day) 30 days for the treatment of active NASH, metformin hydrochloride (Metformin-Teva, LLC Teva Operations Poland) 1000 mg per day, rosuvastatin (Rozuvastatin-Teva, LLC Teva Operations Poland) (5 mg once daily) for 1 month for concomitant type 2 diabetes and hyperlipidemia. Group 2 consisted of patients (38 people) who in addition to similar dietary recommendations and 1 month long therapy, received Quercetin and Povidone (Corvitin (PC NVC "Borshchahivsky CFP", Ukraine) 500 mg in 100 ml of isotonic sodium chloride solution) for 10 days. The mean age of patients was (54.7 ± 3.56) years. Groups of patients were randomized by age, sex, duration of the disease. The comparison group for the presentation of the average reference values of homeostasis indicators consisted of 30 apparently healthy individuals (AHP) of the corresponding age.

The diagnosis of NASH was established in accordance with the unified clinical protocol approved by the order of the Ministry of Health of Ukraine No. 826 dated 06.11.2014, in the presence of criteria for exclusion of chronic diffuse liver disease of viral, hereditary, autoimmune or drug origin as the cause of cytolytic, cholestatic syndromes and on account of the results of ultrasonography (USG) on the ultrasound scanner Ultima PA ("Radmir", Kharkov, Ukraine) with shear wave elastography to determine the stage of liver fibrosis [5], calculation of hepato-renal index (HRI) and biochemical stestotests ("SteatoTest", "ASH" and "NASH-Test" (BioPredictive, France)) – to determine the degree of steatosis of the liver and its nature (alcoholic or non-alcoholic).

Diagnosis of type 2 diabetes was carried out in accordance with the unified clinical protocol approved by the Order of the Ministry of Health of Ukraine No. 1118 dated 21.12.2012. Diagnosis and treatment of CKD was carried out according to the recommendations of clinical guidelines GA "Institute of Nephrology NAMS of Ukraine" (2012). Calculation of glomerular filtration rate (GFR) was performed using a GFR calculator of the Institute of Nephrology of the National Academy of Medical Sciences of Ukraine on the average value of three calculated indicators: creatinine clearance by Cocroft-Golt formula, MDRD and CKD EPI [6]. Determination of DKD stages was carried out according to the classification of Mogensen CE (1983) [9, 6].

The intensity of oxidative modification of proteins (OMP) in serum was determined by the method of Dubinina OE et al. in the modification of Meshchishen IF with regard to the content of aldehyde and ketondinitro-phenylhydrazones (AKDPH) in the blood. The content of LPO products in the blood – isolated double bonds (IDP) in compounds, diene conjugates (DC), ketodienes and conjugated trienes (KCT) – was assayed according to Volchegorsky IA et al., Malonic aldehyde (MA) in blood plasma and Er – according to Vladimirov YuA, Archakov AI. The content of reduced glutathione (RG) in the blood was determined by the titration method according to Travina OV in the modification of Meshchishin IF, Petrova IV. The activity of enzymes of the AODS: glutathione peroxidase (GP) was studied by Meshchishin IF, glutathione-S-transferase (GT) – by Meshchishin IF, catalase – by Korolyuk MA et al. Enzyme activity was calculated per 1 g of Hb. The content of cytokeratin-18 (CC-18) in the blood was assayed by enzyme-linked immunosorbent assay (ELISA) using Elisa reagents. The content of homocysteine in the blood was performed by ELISA using a set of reagents Axis® Homocysteine Enzyme Immunoassay.

Before testing the statistical hypotheses, the analysis of the normality of the distribution of values in randomized samples was performed by determining the coefficients of asymmetry and excess using the Khan–Shapiro–Wilk test. The statistical significance of the difference between the arithmetic mean and its error between the study groups was determined using the bilateral odd Student's t-test. The difference was considered significant at a significance level of $p < 0.05$. Student's t-test was used only in the case of a normal distribution of equal variances of the compared samples, which was checked using Fisher's F-test. In other cases, a nonparametric Mann–Whitney rank test was used to compare the results. The statistical significance of changes in variations over time in the case of normal distribution in the samples was determined by Student's paired test, in other cases – by non-parametric paired T-test of Wilcoxon. For statistical analysis of the obtained results we used Statistica software packages for Windows version 8.0 (Stat Soft Inc., USA), Microsoft Excel 2007 (Microsoft, USA).

Research results and discussion. The analysis of the obtained data showed that before treatment in patients of both groups of comparison a significant degree of OS was established, which was accompanied by a significant accumulation in the

blood of intermediate and final products of LPO and OMP. Thus, before treatment, the content of MA in blood plasma exceeded the reference values by 2.1 times ($p < 0.05$), the content of FRO – by 1.9 times ($p < 0.05$), the content of AKDPH OC – by 2.3 times ($p < 0.05$) (Table 1). At the same time, the state of the antioxidant defense system was significantly unbalanced. Thus, the content of GR in the blood was lower than in AHP by 1.7 times ($p < 0.05$), the activity of G3 – was inhibited by 1.3 times ($p < 0.05$), which explains the high intensity of OS in the subjects patients.

These products of LPO and OMP on the background of significant insufficiency of the ADS system led to the activation of hepatocyte apoptosis. Evidence of this is a significant increase in the content of CC-18 in the blood – by 7.5 times ($p < 0.05$) compared with AHP.

Table 1 – Indicators of oxidative stress intensity, antioxidant protection factors and markers of hepatocyte apoptosis in patients with a combined course of non-alcoholic steatohepatitis, type 2 diabetes mellitus and diabetic kidney disease over time (M ± m)

Indexes	AHP, n = 30	Groups of examined patients			
		Control group, n = 37		Main group, n = 38	
		before	after	before	after
MA plasma, μmol/hour*L	2.22 ± 0.09	4.71 ± 0.09*	3.18 ± 0.07*/**	4.73 ± 0.07*	2.35 ± 0.05**/#
IDP, E220/ml	2.89 ± 0.02	5.53 ± 0.06*	4.76 ± 0.05*/**	5.52 ± 0.08*	3.28 ± 0.04*/**/#
AKDPH OC, U/L of protein	1.37 ± 0.03	3.17 ± 0.08*	2.75 ± 0.04*/**	3.19 ± 0.05*	1.70 ± 0.03*/**/#
GR, μkmol/L	0.93 ± 0.04	0.56 ± 0.05*	0.65 ± 0.04*	0.55 ± 0.06*	0.83 ± 0.02**/#
GP, nmol VG/min*g Hb	152.22 ± 3.46	120.31 ± 5.45*	131.64 ± 5.14*	122.18 ± 5.36*	149.85 ± 3.25**/#
Cytokeratin-18, U/L	57.62 ± 5.37	428.34 ± 17.87*	385.83 ± 15.83*	430.52 ± 18.45*	249.28 ± 12.19*/**/#
Homocysteine, μkmol/L	9.93 ± 0.42	38.27 ± 1.51*	32.62 ± 1.37*	39.23 ± 1.43*	20.42 ± 1.31*/**/#

Note: * – the difference is significant in comparison with the indicator in AHP ($p < 0.05$);
** – the difference is significant in comparison with the indicator before treatment ($p < 0.05$);
# – the difference is significant in comparison with the indicator in patients of the control group after treatment ($p < 0.05$)

Intensive OS and metabolic intoxication also resulted in an increase in the blood content of homocysteine in patients with NASH with DKD – by 3.9 times ($p < 0.05$), which poses a risk of endothelial damage and progression of DKD.

The correlation analysis indicates a strong and medium correlation between the intensity of OS and the content of CC-18 and homocysteine in the blood of patients with NASH with DKD on the background of diabetes mellitus 2 (Table 2), as well as weak and medium relationship with markers of liver damage in NASH and stage DKD ($p < 0,05$).

Analyzing the indicators after treatment should indicate the higher effectiveness of therapy, which additionally contained Quercetin. Thus, significantly increased content of MA in the blood before treatment under the influence of therapy decreased in group 1 by 1.5 times ($p < 0.05$), in group 2 – by 2.0 times ($p < 0.05$). The increased content of the intermediate product LPO IDP decreased by 1.2 and 1.7 times, respectively ($p < 0.05$). The prescribed therapy also had a significant effect on the increased content of AKDPH OC in the blood (2.3 times): the decrease was 1.2 and 1.9 times, respectively ($p < 0.05$). That is, after treatment we found a decrease in the intensity of OS as relative to the oxidation of structural lipids of cell membranes, including endothelium, hepatocytes and podocytes, and relative to structural proteins, due to the established increase in the activity of AODS. This is evidenced by the recovery of more glutathione in erythrocytes: in group 1 – 1.2 times ($p > 0.05$), in group 2 – 1.5 times ($p < 0.05$) and a significant increase in the activity of GP after treatment – only in patients of group 2 1.2 times ($p < 0.05$) (Table 1).

Table 2 – Matrix of correlations of morpho-functional parameters of the liver, kidneys, blood cytokeratin-18 and homocysteine with indicators of oxidative-antioxidant homeostasis in patients with NASH and DKD, DM2 (r, p)

Index	MA	IDP	DC	AKDPH	GR	GP	Catalase
Bilirubin	0.32*	0.43*	0.41*	0.38*	-0.45*	-0.21	-0.23
ALT	0.53*	0.57*	0.58*	0.44*	-0.69*	-0.34*	-0.37*
AST	0.51*	0.53*	0.51*	0.39*	-0.64*	-0.33*	-0.38*
GGT	0.49*	0.44*	0.47*	0.32*	-0.57*	-0.20	-0.25
AP	0.41*	0.43*	0.42*	0.33*	-0.43*	-0.28*	-0.12
Thymol test	0.48*	0.49*	0.47*	0.45*	-0.68*	-0.35*	-0.37*
Albumins	-0.34*	-0.41*	-0.42*	-0.34*	0.59*	0.43*	0.45*
Creatinine	0.58*	0.59*	0.60*	0.63*	-0.67*	-0.50*	-0.53*
GFR	-0.61*	-0.63*	-0.65*	-0.62*	0.62*	0.32*	0.33
Steat test	0.60*	0.62*	0.63*	0.51*	-0.65*	-0.49*	-0.50*
NASH- test	0.63*	0.65*	0.66*	0.52*	-0.68*	-0.53*	-0.56*
Fibrotest	0.54*	0.57*	0.59*	0.57*	-0.67*	-0.55*	-0.57*
CC-18	0.63*	0.68*	0.72*	0.70*	-0.75*	-0.64*	-0.65*
Homocysteine	0.51*	0.53*	0.57*	0.44*	-0.61*	-0.43*	-0.48*

Note: * – statistically significant correlation coefficient ($p < 0.05$)

The obtained research results indicate that a significant decrease in the intensity of apoptosis processes after treatment was registered only in patients of group 2. Thus, the average blood content of CC-18 in patients with NASH with DKD group 2 after treatment significantly decreased by 1.7 times ($p < 0.05$), while in patients with group 1 changes were unlikely.

The effect of the proposed therapy with the addition of Quercetin was also more significant on the content of homocysteine in the blood – the decrease was 1.9 times ($p < 0.05$), and in patients of the control group the indicator only tended to decrease ($p > 0.05$).

Conclusions/Висновки

Comorbid course of nonalcoholic steatohepatitis and diabetic kidney disease in patients with type 2 diabetes mellitus is accompanied by a significant increase in the intensity of oxidative stress, accompanied by an increase in the content of intermediate and final products of lipid peroxidation and oxidative modification of proteins ($p < 0.05$). The damaging effect of oxidative stress in patients with type 2 diabetes leads to activation of hepatocyte apoptosis (7.5-fold increase in blood cytokeratin-18, $p < 0.05$) with the progression of NASH, and an increased risk of endothelial damage due to atherogenesis (3.9 -fold hyperproduction of homocysteine, $p < 0.05$) with the progression of DKD. The use of Quercetin in the treatment of non-alcoholic steatohepatitis and type 2 diabetes with DKD contributes to a significant reduction in the intensity of oxidative stress, increased activity of antioxidant defense factors (reduced glutathione, glutathione peroxidase), resulting in a 1.7-fold decrease and endothelial damage (1.9-fold reduction of homocysteine in the blood).

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Conflict of interest/Конфлікт інтересів

The authors declare no conflict of interest.

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