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MELATONIN EFFECT ON THE OXIDANT STATE AND ENERGY METABOLISM IN THE LIVER MITOCHONDRIA OF RATS UNDER CONDITIONS OF EXPERIMENTAL NEPHROPATHY

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Abstract

Kidney affection results from various diseases, complicates their course and increases the risk of death of a patient. Kidney diseases produce a negative effect on other organs and systems including the liver. The liver is a key organ regulating carbohydrate, lipid and protein metabolism, and provides detoxification of harmful substances. Objective of our work was to study melatonin effect on the oxidant state, energy metabolism and the content of SH- groups in the liver mitochondria of rats under experimental nephropathy induced by folic acid. The state of the liver oxidant system in rats was determined by the intensity of formation of lipid peroxide oxidation products and carbonyl derivatives of the oxidation-modified proteins. To assess the liver energy-supply state the parameters of NADH-ubiquinone oxidoreductase, succinate dehydrogenase, cytochrome c oxidase, H+-ATPase activity, and the content of SH-groups were determined. Nephropathy simulation resulted in the increase of thiobarbituric acid active products (TBA-active products) and carbonyl derivatives of oxidation-modified proteins which are indicative of oxidative stress occurrence. ATP production in the liver mitochondria decreased which might be associated with an inhibiting effect of nephropathy induced by folic acid on the mitochondrial enzymes such as NADH-ubiquinone oxidoreductase, succinate dehydrogenase, cytochrome-c-oxidase, H^+-ATPase. Melatonin introduction during 7 days promoted a considerable decrease of TBA-active products and carbonyl derivatives of oxidation-modified proteins, improved enzymatic activity of I, II, IV and V complexes of the mitochondrial respiratory chain and increased the content of SH-groups. The results obtained confirm a positive effect of melatonin on the oxidation system and energy metabolism in the liver cells, which might be associated with powerful antioxidant properties of melatonin.

Keywords: *nephropathy, liver; mitochondria; oxidation state; energy metabolism; NADH-ubiquinone oxidoreductase; succinate dehydrogenase; cytochrome-c-oxidase; H+-ATPase; melatonin; carbonyl derivatives.*

Introduction

Kidney diseases are one of the most common issues of modern medicine. Kidney affection very often results from various diseases, complicates their course and increases the risk of death of a patient. Patients with COVID-19 in particular are found to suffer from a number of extra-pulmonary complications of different organs and systems including the kidneys as well. Occurrence of acute kidney damage (AKD) in patients infected with COVID-19 ranges from 3 to 6%, increasing to 15-58% in those patients in critical condition [1]. Moreover, increase of diabetes mellitus cases in the world is no less important. It results in the formation of complications such as diabetic nephropathy and renal failure [2]. Diabetes mellitus share among the causes of chronic kidney damage in the world is about 30-47% [3].

In recent years, there is much evidence that oxidative processes play a key role in the development of many diseases. Increasing of reactive oxygen species (ROS) is a key factor of molecular damage identified as oxidative stress. At the same time, the antioxidant system fails to protect against ROS action completely. All the major groups of biomolecules experience damages under effect of free radicals, but the level of oxidative stress is most often defined by the amount of damaged lipid products, protein carbonyls, and mutilated nucleic acids [4].

Pathogenesis of renal pathology is based on the development of oxidative stress accompanied by oxidant-antioxidant imbalance resulting in liver damage. The liver is a key organ playing an important role in metabolism, since it participates in maintenance and regulation of lipid and carbohydrate levels, synthesis of the most valuable proteins and detoxification of toxic compounds of an exo- and endogenic origin. Therefore, the liver is susceptible to oxidative damages [5, 6].

The mitochondrial respiratory chain is very often the main source of ROS formed as a by-product of electron transport [7,8].

It stipulates the necessity to search of the means of effective and safe correction of nephropathy of different etiologies. Lately scientists and doctors have become interested in melatonin as a neuroendocrine hormone with antioxidant properties. Its highest concentration was found in mitochondria [9]. A positive melatonin effect was found in case of various pathologies including diabetes mellitus as well [10].

Therefore, objective of our work was to study melatonin effect on the oxidant state in the liver cells, energy metabolism and the content of SH-groups in the liver mitochondria of rats under experimental nephropathy.

Methods

The experiment was carried out on 127 albino mature male rats with the body weight of 0,16 – 0,18 kg. All the animal experiments were performed according to the European guidelines for the use and care of animals for research in accordance with the European Union Directive of September 22, 2010 (2010/63/UE).

Nephropathy was simulated by means of a single introduction of folic acid into the peritoneum (Sigma-Aldrich, USA) in the dose of 250mg/kg of the body weight [11]. The animals were divided into 5 groups: 1st one – control animals; 2nd – experimental animals with simulated nephropathy (3rd day); 3rd group – animals receiving melatonin(Sigma-Aldrich, USA) with underlying simulated experimental nephropathy at the dose of 10 mg/kg of the body weight during 3 days into the stomach; 4th one – animals with simulated nephropathy (7th day); 5th group – animals with experimental nephropathy receiving melatonin in the dose of 10 mg/kg of the body weight every day during 7 days.

The liver oxidant system state of rats was determined by the content of thiobarbituric acid active products (TBA-active products) and content of protein carbonyls, which are irreversible markers of protein oxidation. To assess the state of the liver energy supply the parameters of NADH-ubiquinone oxidoreductase, succinate dehydrogenase, cytochrome c oxidase, H+-ATPase activity, and the content of SH-groups were determined.

The content of TBA-active products in the post-mitochondrial supernatant of the liver tissue homogenates was determined by the reaction between Malone dialdehyde and thiobarbituric acid which at a high temperature and acid pH results in the formation of stained trimethine complex [12]. The maximum of the complex absorption is 532 nm.

The content of TBA-active products was calculated considering molar extinction1,56×10^5 M^{-1}cm^{-1}. The results were presented in μM /g of the liver tissue.

Oxidative protein modifications (OMP) represented by methods to detect protein carbonyls in the post-mitochondrial supernatant of the liver tissue homogenates were determined by the reaction with 2,4-dinitrophenylhydrazine with formation of hydrazones of a specific spectrum of absorption [13]. The degree of the oxidation-modified proteins was estimated by the amount of aldehyde and ketonic groups formed. Aldehyde and ketonic derivatives of the neutral character are found with the wave length of 370 nm, and of the alkaline character – with 430 nm. Considering the molar extinction coefficient (2,1×10^4 M^{-1}см$^{-1}$) the content of derivatives of 2,4-dinitrophenylhydrazine of a neutral and main character were found, and expressed in nmol of carbonyl derivatives per 1 mg of protein.

The activities of NADH-ubiquinone oxidoreductase, succinate dehydrogenase, cytochrome-c-oxidase, H+-ATPase and the content of SH-groups were examined in the mitochondria of the liver, as reported previously [14] using the spectrophotometer Agilent Cary 60.

Briefly, mitochondria were isolated by differential centrifugation in the isolation buffer [15]. The rats' liver was washed with a cooled 0.9% solution of KCl (2-4 °C), chopped and homogenized in 10 times the volume of buffer pH 7.4, which contains: sucrose – 250 mmol/l, tris-HCl – 25 mmol/l and 1mM EDTA. The homogenate was centrifuged at 700 g for 10 min (4 °C), and the supernatant – at 11 000 g for 20 min (4 °C) using the centrifuge HERMLE Labortechnik. The precipitate was re-suspended in 5 ml of the same buffer (without EDTA) and centrifuged again under the same conditions and used immediately in experiments.

NADH-ubiquinone oxidoreductase activity was determined by NADH oxidation rate. 0.02 ml of mitochondrial fractions and 100 μM NADH were added to the test tubes containing 2 ml of 0.02 M tris phosphate buffer (pH 7.4). Measurements were carried out at 340 nm per decrease in optical density for 2 min with an interval of 20 sec [16]. The activity of NADH- ubiquinone oxidoreductase was calculated on the basis of molar extinction 6.22 • 10^3 M^{-1} cm^{-1}.

The succinate dehydrogenase activity was measured based on restoration of potassium ferricyanide ($K_3[Fe(CN)_6]$), to colorless potassium ferrocyanide ($K_4[Fe(CN)_6]$), by the action of succinate dehydrogenase [17]. The suspension of mitochondria (0.2 ml) was added to incubation solution (10 mM phosphate buffer (pH 7.8), 5 mM succinic acid, 25 mM EDTA, 150 mM sodium azide). Samples were incubated at room temperature for 5 min. The reaction was initiated by addition of 25 mM potassium ferricyanide solution to the samples. Samples were incubated for 10 min at 30 °C. After incubation, the reaction was stopped by lowering the sample temperature to 0 °C. In the control samples containing all components of the incubation mixture, CCl_3COOH was added before the introduction of the mitochondrial suspension. After arrest of the reaction, the samples were analyzed at λ 420 nm. The activity of the enzyme is proportional to the amount of ferricyanide.

The method of cytochrome-c-oxidase determination is based on the ability of the enzyme to oxidize dimethylparaphenylidine and α-naphthol with the formation of indophenol blue. Its concentration is proportional to cytochrome oxidase activity [18]. 1 ml of the reaction substrate (α-naphthol, paraphenyldiamine and sodium carbonate in a ratio of 1:1:1) was injected into the test tubes. 0.1 ml of the mitochondrial fraction suspension (and 0.1 ml of H_2O in the control test tube) was added to the mixtures. After incubation (37°C, 30 min) 3 ml of ethanol was added to each test tube. Samples were centrifuged for 10 min at 6000 g. The samples were measured by spectrophotometric method at λ 550 nm.

H+-ATPase activity was evaluated according to the accumulation of inorganic phosphate [19]. The activity was determined in an incubation solution containing 400 μmol of tris-HCl (pH 7.4), 5 μmol of ATP disodium salt, 7.5 μmol of $MgSO_4$,·5·10^{-2} μmol of 2,4-dinitrophenol, 7.5 μmol of $CaCl_2$, 120 μmol of NaCl, 20 μmol of KCl. The reaction was initiated by addition of 50 μl of a mitochondria suspension. Samples were incubated for 15 min at 37 °C. The reaction was arrested by addition of 1 ml of 10% CCl_3COOH. The contents of Pi were determined by means of the colorimetric method.

The protein level was determined by Lowry's method [20].

The content of SH-groups was determined by the method [21] based on the interaction of Elmann reagent (5,5′-dithio-bis(2-nitrobenzoic acid) with SH-groups. For determination of the total SH-group level, the reaction mixture composed of 0.2 ml of the examined suspension of mitochondria, 0.1 ml of NaOH, 3.7 ml of phosphate buffer (pH 8.0), 0.1 ml of DTNB, was incubated for 10 min at room temperature and then the sample was centrifuged at 10 000 g for 10 min using the centrifuge HERMLE Labortechnik. The content of SH-groups was calculated using a molar extinction coefficient of 11400 M^{-1} cm^{-1} and expressed in nmol/mg protein.

The type of distribution was estimated using the Shapiro-Wilk test. Significant differences between the groups were evaluated by means of the nonparametric Wilcoxon criterion. $P < 0.05$ was considered statistically significant [22]. All the results in figures are represented as median minimum-maximum values (Me[min-max]), the results in Table are represented as mean ± standard error of the mean (M±SEM).

Results

We have found intensification of lipid and protein oxidation processes in the liver cells (Table 1), which is evidenced by an increased content of TBA-active products on the 3rd and 7th days of experimental nephropathy by 19,9%, and 38,8% respectively; as well as the content of carbonyl derivatives of protein oxidation of a neutral character by 42,6% and 16,1% respectively in comparison with the control values. Increase in the content of carbonyl derivatives of protein oxidation of the alkaline character was found to be 17,5% on the 7th day of nephropathy appearance.

OMP increase results from imbalance disorders between the processes regulating synthesis and oxidation of proteins, decreased activity of protease selectively breaking down oxidized forms of proteins. Protein carbonyls represent the most frequent and usually irreversible oxidative modification affecting proteins and are chemically stable in contrast to lipid peroxidation products that are removed within minutes [23].

One of the causes of OMP changes can be lipid peroxidation uncontrolled intensification. Lipid peroxidation products and Malone dialdehyde in particular, are known to cause degradation of lysine protein residues reacting with them followed by the formation of various cytotoxic compounds. OMP intensification can result from functional disorders of the protective antiradical systems. In addition, protein carbonyls are formed early during oxidative stress conditions and are not a result of one specific oxidant, thus they can be called a marker of overall protein oxidation. Increase in the content of TBA-active products and oxidation-modified proteins in the post-mitochondrial fraction of the liver cells of rats with experimental nephropathy are indicative of stimulation of free radical oxidation processes.

After melatonin introduction the parameters of TBA-active products and carbonyl derivatives of protein oxidation of the neutral character decreased on the 3rd day by 23,9% and 22,3%, on the 7th day – 31,3% and 21,5% respectively in comparison with the group of animals afflicted with nephropathy. Decrease of carbonyl derivatives of protein oxidation of the alkaline character by 25,2% was found on the 7th day of melatonin introduction as well. Therefore, melatonin introduction is able to decrease the degree of protein and lipid peroxidation.

Oxidative stress occurs when the mechanisms of the antioxidant protection fail to neutralized free radicals in the cell. This imbalance between production of oxidized molecules and antioxidant protection results in accumulation of ROS, that oxidize and cause damage of lipids, proteins and DNA molecules [24, 25].

Since the mitochondrial respiratory chain is a source of ROS, we have examined the activity of mitochondrial enzymes in the liver cells of rats.

Activity of NADH-ubiquinone oxidoreductase (Figure 1) in the mitochondria of the liver cells of rats with experimental nephropathy induced by folic acid decreased by 9,1% on the 3rd day and 7,0% on the 7th day of the experiment in comparison with the control group of animals. Respiratory Complex I (NADH-ubiquinone oxidoreductase) is one of the biggest enzymatic complexes in oxidative phosphorylation and the point of electron entrance into the mitochondrial respiratory chain. It oxidizes NADH, reduces ubiquinone and carries protons

through the internal mitochondrial membrane, promoting generation of the proton driving force essential for ROS production. Moreover, complex I is the main generator of intracellular ROS [26, 27]. Several pathologies are known to be associated with elevated production of superoxide by defective complex I [28]. The data obtained are indicative of dysfunction in the complex I of the respiratory chain.

We have determined (Figure 2) decrease of succinate dehydrogenase activity in the mitochondria of the liver cells of rats with experimental nephropathy on the 3rd day of the experiment by 26,8% and on the 7th day – by 16,7% in comparison with the parameters of the control group of animals, which might be indicative of disorders in the structure of II respiratory complex.

Respiratory Complex II (succinate dehydrogenase) is a protein complex participating in oxidation of succinate to fumarate and ubiquinone reduction, and in this way combining the cycle of tricarboxylic acids with electron transport chain. At the same time, succinate dehydrogenase is the only respiratory complex that does not participate in transportation of protons through the internal membrane during the whole catalytic cycle. For a long time succinate dehydrogenase was not considered to participate in the formation of free radicals, but the latest studies [25, 29] demonstrate an opposite view. Although in comparison with the parameters of ROS formation in I or III complexes, their formation in II complex is usually inconsiderable.

Activity of cytochrome c oxidase and H^+-ATP-ase (Table 2) in the mitochondria of the liver cells of rats with experimental nephropathy decreased by 42,0% and 37,5% on the 3rd day and by 37,9% and 33,1% on the 7th day of the experiment in comparison with the control group of animals.

Respiratory Complex IV (cytochrome c oxidase) transfers electrons from cytochrome-c-oxidase to the terminal electron acceptor O_2 to generate H_2O [25]. In our opinion, reduced activity of cytochrome-c-oxidase is associated with dysfunction of I and II complexes, resulting in possible "escape" of electrons exactly on these portions of the respiratory chain.

Electron flow is associated with generation of proton gradient through the internal membrane, and the energy accumulated in the proton gradient is utilized by the final complex V for ATP production [24]. With nephropathy induced by folic acid, effect of phosphorylation decreased during oxidation of both: $NADH+H^+$ and $FADH_2$ which influenced on ATP production respectively.

Inhibition of the complexes I, II, IV and V resulted not only in ROS induction but decreased ATP level in the cells due to interruption of the mitochondrial respiration [30].

Moreover, we have found changes in the content of free SH-groups in the mitochondrial fraction of the liver of rats with experimental nephropathy. The content of SH-groups decreased by 30,9% (3rd day) and by 33,2% (7th day) in comparison with the control group of animals.

SH-containing compounds undergo oxidation first and foremost. It protects other functional groups and molecules against oxidation. In addition, SH compounds are important components in maintenance of oxidation-reduction homeostasis in the cells and tissues. Changes in the content of SH-groups with experimental nephropathy might be associated with a high intensity of ROS generation which is indicative of intensification of free radical processes in the liver mitochondria.

Melatonin introduction influenced on the oxidation of substrates of I and II complexes.

Melatonin introduction promoted increased activity of NADH-ubiquinone oxidoreductase in the mitochondria of the liver cells of rats by 7,9% on the 7th day in comparison with the parameters of animals afflicted with nephropathy. Succinate dehydrogenase activity increased by 24,6% on the 3rd day and by 16,8% on the 7th day in comparison with animals with experimental nephropathy. The results showed that on the 3rd day melatonin increases the activity of cytochrome-c-oxidase and H^+-ATP-ase in the mitochondria of the liver cells by 78,6% and 25,3%, and on the 7th day – by 44,8% and 24,5% respectively in comparison with animals with experimental nephropathy. Though, an increased activity of H^+-ATP-ase differed significantly from that of the control.

Increasing content of SH-groups with melatonin correction was found on the 7th day by 14,5% in comparison with the group of animals with nephropathy, but still they do not reach the parameters of the control group.

Melatonin introduction during 7 days results in improvement of enzymatic activity of I, II, IV and complexes, as well as in changes of oxidation-reduction state of protein SH-groups of the mitochondrial respiratory chain in the liver cells, which demonstrates an important effect of melatonin produced on the energy metabolism, and can be considered as a compensatory reaction of the body to oxidative stress caused by nephropathy. In addition to a direct action on free radicals, melatonin is able to improve efficacy of electron transport chain preventing "escape" of electrons and reducing the risk of oxidative stress occurrence [31].

Discussion

Our data obtained are proved by the results of other researchers concerning the fact that mitochondrial dysfunction causes disorders of oxidative phosphorylation, apoptosis and excessive formation of ROS resulting in disorders of energy metabolism [32, 33]. Dysfunctional mitochondria lead to the "escape" of harmful active oxygen forms occurring on the portions of I, III complexes and II complex partially. [7, 26]. Occurring oxidative stress results in inactivation of mitochondrial enzymes followed by a reduced level of ATP formation [34].

Melatonin is a rather lipophilic compound able to penetrate through the cellular membranes reaching intracellular organelles including mitochondria. Recent studies have found that mitochondrial membranes possess special proteins-carriers promoting quick melatonin absorption through the organelles against the gradient of concentration [35]. Melatonin is suggested to interact with the complexes of the mitochondrial respiratory chain giving and taking electrons, and in this way promoting the electron flow. Its antioxidant action and possible interaction with mitochondrial complexes possessing synergic action is able to improve the work of the respiratory chain and provide ATP adequate production [35].

Molecular damage caused by especially highly reactive ROS can be controlled either by means of preventing formation of their precursors or by means of their removal immediately after their formation [35]. Melatonin is able to decrease poorly reactive oxygen forms both at the expense of its direct antioxidant properties catching free radicals on the level of the mitochondrial chain of electron transition, and at the expense of indirect ones – stimulating activity of antioxidant enzymes.

Melatonin is confirmed to stimulate activity of antioxidant enzymes, promotes efficacy of the electron-transport mitochondrial chain resulting in decrease of the "escape" of electrons and formation of free radicals [36]. Direct antioxidant and "radical catching" properties of melatonin are stipulated by its structure. Its structure contains the aromatic indole ring rich in electrons, which makes it a powerful donor of electrons and results in a considerable decrease of oxidative stress [37].

Conclusions

Simulation of nephropathy resulted in oxidative stress occurrence characterized by an increased content of TBA-active products and carbonyl derivatives of oxidation-modified proteins. The ability of mitochondria to oxidize substrates, generated by NADH and FAD, decreased with nephropathy.

In the course of the experiment reduced ATP production in the mitochondria of the liver was found that might be associated with an inhibiting effect of nephropathy induced by folic acid on the mitochondrial enzymes. Therefore, the activity of respiratory complexes decreases with nephropathy, which is caused by oxidative damage of proteins that constitute the base of the internal membrane of the mitochondrial respiratory chain, and lipids participating in stabilization of the respiratory complexes in the membrane.

A protective action of melatonin is demonstrated in case of nephropathy induced by folic acid against oxidative stress and mitochondria depended destructive processes in the liver.

Melatonin introduction decreased tissue degradation which is evidenced by reduced intensity of lipid peroxidation products and the content of carbonyl derivatives. Melatonin improves the ability of mitochondria to oxidize substrates generated by NADH and FAD, produces a positive effect on cytochrome-c-oxidase and H^+-ATP-ase activity, which might promoted improvement of mitochondrial adhesion with oxidative stress caused by nephropathy. Due to its antioxidant properties melatonin is able to improve functioning of the respiratory complexes at the expense of absorption of free radicals, reduction of oxidative stress level,

apoptosis restriction, maintenance of efficacy of the respiratory chain complexes and providing an adequate level of ATP formation occurring with experimental nephropathy.

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Table 1. The state the of the liver oxidant system of rats with experimental nephropathy under conditions of melatonin correction

	Control, n=36	Nephropathy, 3rd day, n=25	Nephropathy +melatonin, 3rd day, n=25	Nephropathy, 7th day, n=24	Nephropathy + melatonin, 7th day, n=24
TBA-active products, μM/g of the tissue	21,04±0,65	25,23±1,60**	19,21±1,11#	29,20±3,31**	20,07±1,79##
carbonyl derivatives of protein oxidation of the neutral character, nmol /mg of protein	3,05±0,11	4,35±0,33*	3,38±0,17#	3,54±0,19**	2,78±0,13#
carbonyl derivatives of protein oxidation of the alkaline character, nmol /mg of protein	2,74±0,12	3,05±0,17	2,50±0,12#	3,22±0,21**	2,41±0,10#

The results are represented as mean ± standard error of the mean (M±SEM). *– statistically significant differences in comparison with the parameters of the control group of animals; # – statistically significant differences in comparison with the parameters of the group of animals with nephropathy; p<0,01; ** - statistically significant differences in comparison with the parameters of the control group of animals; ## – statistically significant differences in comparison with the parameters of the group of animals with nephropathy on the 7th day of the experiment, p<0,05.

Table 2. Activity of cytochrome-c-oxidase (CO) and H^+-ATP-ase in the mitochondria of the liver cells of rats with experimental nephropathy

	Control, n=36	Nephropathy, 3rd day, n=25	Nephropathy +melatonin, 3rd day, n=25	Nephropathy, 7th day, n=24	Nephropathy + melatonin, 7th day, n=24
cytochrome-c-oxidase, nmol/ min*mg of protein	0,0169±0,00218	0,0098±0,00169*	0,0175±0,00321#	0,0105±0,00184*	0,0152±0,00137#
H^+-ATP-ase, mmol P/ min*mg of protein	4,69±0,13	2,93±0,12*	3,67±0,17*,#	3,14±0,13*	3,91±0,18*,#

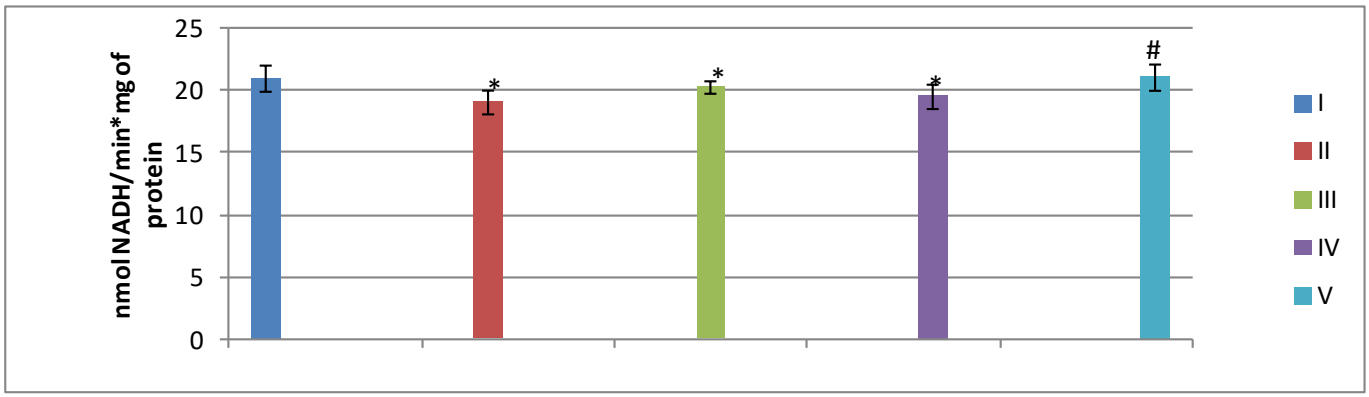
The results are represented as mean ± standard error of the mean (M±SEM). *– statistically significant differences in comparison with the parameters of the control group of animals; # – statistically significant differences in comparison with the parameters of the group of animals with nephropathy; p<0,01.

Table 3. Content of SH-groups in mitochondria of the liver cells of rats with experimental nephropathy

	Control, n=36	Nephropathy, 3rd day, n=25	Nephropathy +melatonin, 3rd day, n=25	Nephropathy, 7th day, n=24	Nephropathy + melatonin, 7th day, n=24
SH-groups, nmol/mg of protein	9,01±0,21	6,23±0,37*	6,65±0,36*	6,02±0,42*	6,89±0,42*,#

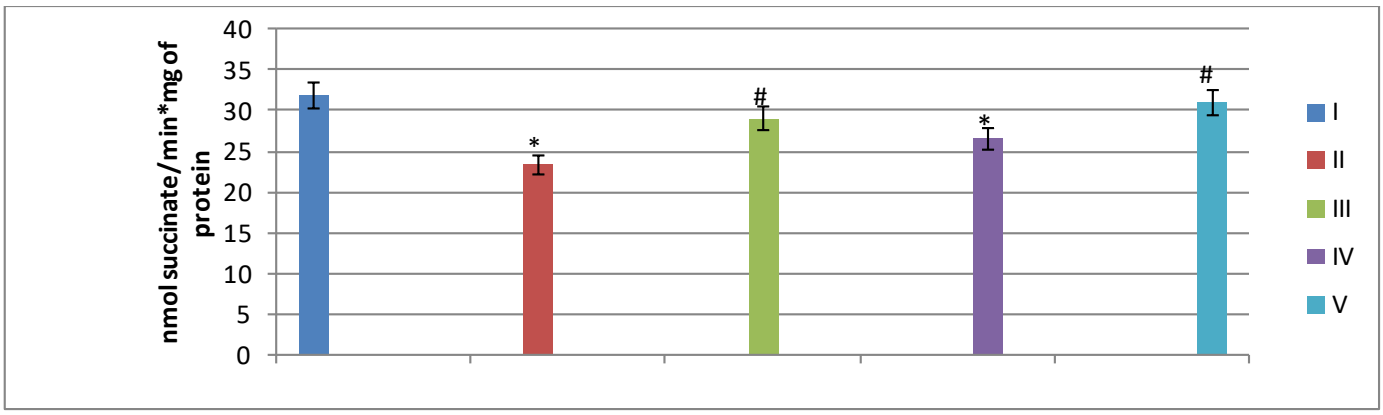
The results are represented as mean ± standard error of the mean (M±SEM). *– statistically significant differences in comparison with the parameters of the control group of animals; # - statistically significant differences in comparison with the parameters of the group of animals with nephropathy on the 7th day of the experiment, p<0,05.

Figure 1. Activity of NADH- ubiquinone oxidoreductase in the mitochondria of the liver



I – control (n=36), II – nephropathy (3 day (n=25)), III – nephropathy + 3 days of melatonin introduction (n=25), IV - nephropathy (7 day (n=24)), V – nephropathy + 7 days of melatonin introduction(n=24); * - p<0.01, relative to control, # - p<0.01, relative to animals with nephropathy. M±m; the data are presented as mean±SEM.

Figure 2. Activity of succinate dehydrogenase in the mitochondria of the liver



*I – control (n=36), II – nephropathy (3 day (n=25)), III – nephropathy + 3 days of melatonin introduction (n=25), IV - nephropathy (7 day (n=24)), V – nephropathy + 7 days of melatonin introduction(n=24); * - p<0.01, relative to control, # - p<0.01, relative to animals with nephropathy.* M±m; the data are presented as mean±SEM.