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RESEARCH ARTICLE

LOW MOLECULAR WEIGHT COMPONENTS OF COLOSTRUM REGULATE THE ACTIVITY OF CELLULAR COMPONENT OF THE IMMUNE SYSTEM IN ANIMALS WITH CU-INDUCED LIVER FIBROSIS

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ABSTRACT

It has been study the effect of low molecular weight components of colostrum (LMCC) on some parameters of immune system and prooxidant-antioxidant system on the model of Cu-induced liver fibrosis. For the induction of liver fibrosis in rats were injected copper sulphate intraperitoneally three times successively at a dose 33% of lethal in rats. It is shown that the most marked changes in the initial stages of development of fibrosis manifested at the level of pro-antioxidant system, which is correlated with a change in the pattern of immunological parameters. These changes are manifested in the inhibition the growth of animals and decreasing their body temperature.Administration LMCC animals with liver fibrosis was accompanied by "removing" oxidative stress, activating of cellular immunity and restoring the growth rate and body temperature of the experimental animals.

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INTRODUCTION

The immune system is one of the regulatory systems of the body, which ensures elimination of emerging pathologies and provides a general regulation of homeostasis (P.C. Calder, 2013; C. Castelo-Branco and I. Soveral, 2014; S. Hussain *et al*., 2012). It is commonly known that the number of patients with liver diseases (hepatitis, fibrosis, cirrhosis) has been steadily increasing (M. Blachier*et al*., 2013; Fu-Sheng Wang *et al*., 2014). That fact is attributed to changes in diet, use of large amounts of medical drugs, alcohol, an increased content of toxic compounds in the environment, heavy-m*et al* ions particularly (W.R. García-Niño *et al*., 2014; Minjun Chen *et al*., 2015; Jürgen Rehm *et al*., 2013). It has been demonstrated that an outcome of development of a number of liver pathologies may be inversive or irreversible and lead to development of cirrhosis and to complete loss of body functions (J.HLefkowitch, 2016; A. Pellicoro *et al*., 2012). It is still not completely clear as to what factors determine the strategy for development of fibrosis. Previously it has been demonstrated that a repeated sequential administration of copper compounds to experimental animals at a dose corresponding to 33% of the lethal, would induce liver fibrosis in them (A.I. Bozhkov *et al*., 2010). Research of activity of the immune system in animals with Cu-induced liver fibrosis is of great interest both for understanding its role in liver fibrosis development strategy and for understanding of the role of endogenous factors in regulation of the immune response. It is known that colostrum is one of unique and complex factors that regulate the activity of the immune system (M. Yang *et al*., 2015; M. Rathe *et al*., 2014; Ebenezer Satyaraj*et al*., 2013). It contains three times more solids compared to milk (Chantal Farmer, 2015), large amounts of vitamins, minerals, and other compounds (Brian A *et al*., 2016; P. Sacerdote *et al*., 2013). In addition to that the colostrum is rich with various cytokines that affect the immune system activity (L. Zhang *et*

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al., 2015). However, individual components of the immune system after intake of colostrum against the backdrop of fibrosis development still remain insufficiently researched. In addition, it has been demonstrated that the composition of colostrum includes a so-called "high molecular weight" immunoglobulins which possess allergenic effect (M. Conneely *et al*., 2013), that makes its direct administration difficult. In connection with that, an impact of low molecular weight components of colostrum (LMCC) on neutrophil phagocytic activity indexes, a content of circulating immune complexes (CIC), of average molecular weight peptides (AMWP), and activity of NADFH-dependent intracellular neutrophil enzymes in animals with Cu-induced fibrosis and with fibrosis treated with low molecular weight components of colostrum (LMCC) at various doses, as well as a lipid hydroperoxide content, a glutathione peroxidase activity, dynamics of the mass and the body temperature of experimental animals have been researched.

MATERIALS AND METHODS

The experiment was performed on 3-month old male rats of Wistarline. All the animals were divided into 3 groups (Fig. 1). The animals were kept under a standard vivarium mode, had free access to water. Throughout the experiment, the body weight and animals rectal body temperature were measured by means of a thermometer MicroTherma 2T Hand Held (Braintree scientific, Inc., USA).

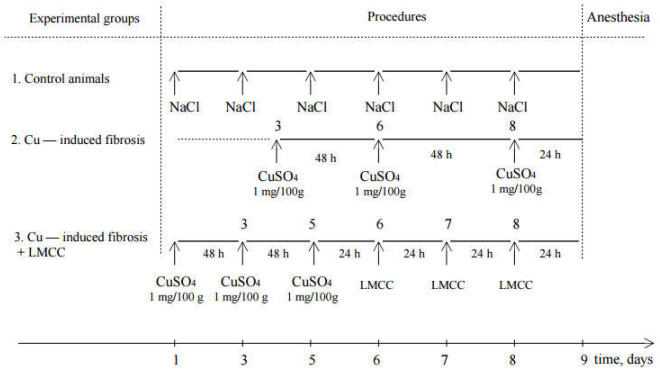


Figure 1. The diagram shows sequential administration of copper sulfate 1 mg/100 g of the body weight of an animal at 48-hour intervals between the doses for induction of fibrosis (option 2), and the same with a subsequent administration of threefold low molecular weight components of colostrum (LMCC) at 24-hour intervals between the administrations (option 3), and the same manipulations of saline injection instead of copper ions to intact control (option 1)

A solution of copper sulphate ($CuSO_4 \cdot 5H_2O$) was prepared in physiological saline (0.95% NaCl) immediately before the administration. The injection of the solution ($CuSO_4 \cdot 5H_2O$) was executed intraperitoneally at the dose of 1 mg/100 g of the liver weight, always after 9.00 - 10.00 am. All procedures with the animals were carried out in accordance with observance of the bioethical norms (Council Directive 86/609/EEC, 1986).

Method for determination of phagocytic activity of peripheral blood neutrophils

The method is based on the ability of polymorphonuclear leukocytes to bind to its surface, absorb and digest the microbial test culture (Saccharomyces cerevisiae). This method allows for determination of barrier functions of primary resistance factors. The material for the research was a heparinized blood of experimental animals. To assess the phagocytic activity of granulocytic neutrophils equal quantities of 400 microliters of a heparinized whole blood and *Saccharomyces cerevisiae* cell inoculum at a concentration of 1% were mixed in 2 sterile centrifuge tubes and incubated at 37°C for 30 and 120 min. Cell smears were fixed in 96% ethanol and stained according to Romanowsky-Giemsa staining for 20 min. 200 cells were counted with a light microscope (Microscope Primo Star Care Zeiss Surhou Co. Ltd.) at a magnitude of x900. The number of cells which absorbed the test culture was counted in the analysis of 200 cells and the number was expressed as a percentage –the phagocytic index (PI). The average number of *Saccharomyces cerevisiae* cells, which has been absorbed by one cell –the phagocytic number (PN) was determined and expressed in arbitrary units. To determine the endocytosis intensity the index of phagocytosis completeness (IPC) was calculated by the ratio of the PN in 30 min. to the PN in 120 minutes. (Muniz-Junqueira, M.I. *et al*., 2003).

A recovery of nitro blue tetrazolium (NBT-test) is the method for determination of an overall redox activity of neutrophils in the test

The method is based on the ability of neutrophils to absorb nitro blue tetrozolium and restore its granules in insoluble diformazan in the form of blue granules under the influence of superoxide anion generated in the NADPH oxidase reaction initiating the phagocyte stimulation process. Heparinized whole blood of experimental animals was the material for the research. To assess the enzymatic activity of granulocytic neutrophils equal amounts of 50 µl, heparinized whole blood and nitro blue tetrazolium (NBT) were mixed in two sterile centrifuge tubes, then 50 µl. of 0.15M phosphate buffer with pH=7.2 - an indicator of the level of spontaneous NBT test (SP) were added in the first tube, and 50 ml. of 0.5% opsonized zymosan suspension - stimulated NBT test (ST) were added in the second tube. The samples are incubated for 30 minutes at 37°C, then centrifuged for 10 minutes at 1000 g, the supernatant fluid was collected, the precipitate was resuspended. Cell smears are fixed with 96% ethanol and stained with 0.1% neutral red for 40 min. 100 cells were counted with a microscope (Microscope Primo Star Care Zeiss Surhou Co. Ltd.) at a magnitude of x900. A number of cells that contain inclusion of diformazan or its precipitation ware determined (Freeman, R. and King, B., 1972).

Method for determination of concentration of circulating immune complexes

The method is based on a selective precipitation of antigen-antibody complexes in polyethylene glycol (PEG 6000), followed by a photometric determination of the precipitate density. A number of circulating antigen-antibody immune complexes in serum was determined. To determine the amount of circulating immune complexes (CIC) 100 µl of blood serum were introduced in each of the two chemical test tubes, then 200 µl of 0.1M borate buffer with pH=8.4 were added in each of the two chemical test tubes. 2.7 ml of 0.1M borate buffer with pH = 8.4 was added in the control sample and 2.7 ml of 3.75% PEG solution with the molecular weight of 6000 was added in the test tube. The samples were incubated for one hour at 20°C. The optical dense of the control and experimental samples were determined with a

spectrophotometer at λ=450 nm, against borate buffer. The content of the CIC was expressed in arbitrary units (Lambert P.H. *et al*., 1978).

Method for determination of a concentration of average molecular weight peptides

For determination of average molecular weight peptides, 300 µl of blood serum were added to a centrifuge tube and then 150 µl of 10% TCAwere added. The high molecular weight proteins were pelleted for 30 min at 3000 g. We determine the samples optical dense of the supernatant fluid with a spectrophotometer at λ=254 nm against distilled water. The content of the AMWP was expressed in arbitrary units (Gabrieljan N.I., 1985).

Method for determination of lipid hydroperoxide (LH) in blood serum

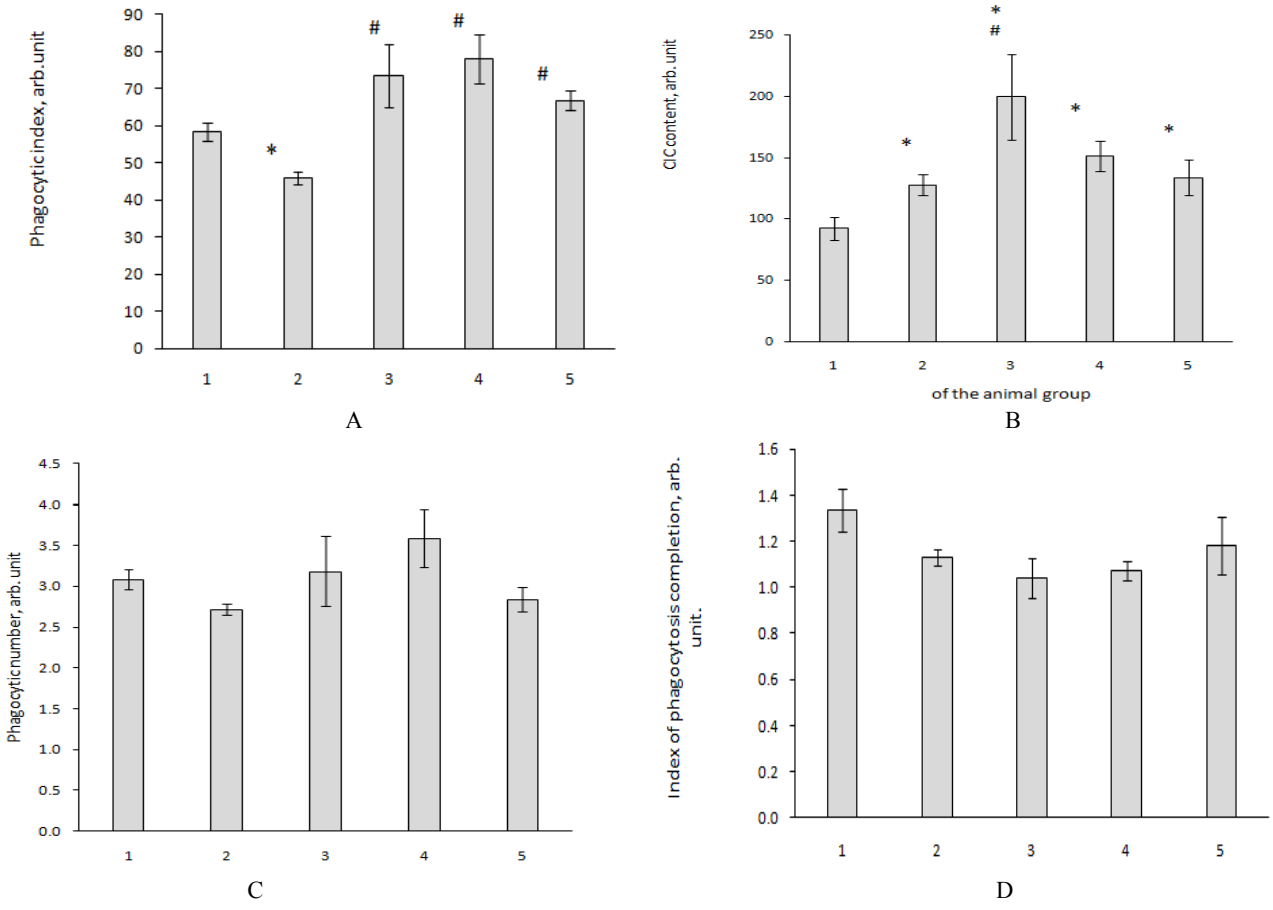
AnLH content in blood serum was determined with the method (Asakawa T., 1980). The absorption spectrum of the stained product was recorded on a double-beam spectrophotometer Specord UV VIS, later with the extinction difference was measured at 535 and 520 nm (Massie H. R. 1983). The lipid hydroperoxide content is expressed as equivalent amounts of MDA using the molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$. In some experiments, a calibration curve with 1,1,3,3-tetraetoksipropan was used as the standard.

Determination of the activity of Glutathione peroxidase (GPx) (EC 1.11.1.9)

The GPx activity was determined in blood serum by spectrophotometry at λ = 340 nm according to the method (Paglia D. E., 1967) with minor alterations (Lankin V. Z., 1976) in 50 MW K^+,Na^+-phosphate buffer (pH 7.4), that contains 1 MW EDTA, 0.15 MW NADPH, 1 unit of glutathione reductase of yeast, 0.2 % Triton X-100 and 3 MW of azide Na for inhibition of keto-acyl thiolase (KAT). Cumenehydroperoxide was added at the concentration of 1.2 MW, hydrogen peroxide – 0.4 MW. Temperature – 37 °C. The GPx activity wasexpressed in nmoleNADPH/min for mg of protein or ml of serum taking into consideration the molar extinction coefficient $6.22 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$. Statistical analysis of the results. All experiments were repeated at least 3 times. At least 6 animals were in each experiment point. An average value, a standard error, and significant differences were determined in comparison to the intact control and with Cu-induced liver fibrosis, a standard edition of Statistica 10 software was used (Glantz, S., 1996).

RESULTS

The amount of neutrophils that devoured the bacteria in an*in vitro* system (phagocytic index - PI) was reduced by 22% in the animals with Cu-induced liver fibrosis in comparison with the intact control (Fig. 2 A).



* – p < 0.05 compared to intact control;
# – p < 0.05 in comparison with fibrosis

Figure 2. Phagocytic Index (A), content of circulating immune complexes (B), phagocytic number (C), index of phagocytosis completion (D) in the control animals (1), in the animals with Cu-induced fibrosis (2), in the animals with fibrosis that were administered LMCC three times at the dose of 0.1 g/100 g of the body weight of the animal(3), also at the dose of 0.05 g (4) and at the dose of 0.01 g (5).

Those animals had an increased content of circulating immune complexes (CIC) by 38% in comparison to the intact control (Fig. 2 B). The absorption capacity of phagocytes (phagocytic number - PN) and their digestive capacity (index of phagocytosis completion - IPC) were not different from the intact control (Fig. 2). Therefore, at early stages of development of Cu-induced liver fibrosis a specific pattern of values of cellular components of immune system and CIC. A decrease in the number of active phagocytes and as a consequence an increase in the content of CIC is characteristic of that pattern. In the case when animals with Cu-induced liver fibrosis were injected with a low-molecular components of colostrum (LMCC) 3 times at intervals between the doses of 24 hours at the dose of 0.1 g/100 g of the animal weight, then the phagocytic index increased by 40% in comparison to the group with fibrosis and by 25% in comparison with the intact control. (Fig. 2 A). The effect of agitation of the phagocytic index remained even when the LMCCdose was reduced twofold and 10-fold (Fig. 2 A). However, the phagocytic number and the index of phagocytosis completion remained unchanged in comparison to fibrosis (Fig. 2 C, D). Administration of LMCC at the dose of 0.1 g/100 g of the body weight to the animals with fibrosis increased the CIC content by 55% in comparison with the fibrosis (Fig. 2 B). In case when the LMCC doses were reduced down to 0.05 and 0.01 g/100 g of the body weight of the animal, the content ofCIC in the blood serum was also reduced and was not different from that in the animals with fibrosis, but it remained higher than in the intact control (Fig. 2 B).Therefore, LMCC stimulated the phagocytic index and increased the content of CIC. As it is known, CIC - high molecular weight albuminous compounds which are formed as the result of formation of immune "antigen-antibody" complexes. The increase of CIC reflects pathogenic mechanisms of several autoimmune diseases and multiple organ dysfunctions. However with a short-term antigenemia it does not lead to development of pathologies.

Increase in CIC in animals with Cu-induced fibrosis treated with LMCC can be interpreted on the one hand, as a manifestation of the immunogenicity of colostrum components, and perhaps as an increase in the rate of formation of immune complexes after a development of fibrosis, with a possible result in recovery of a decreased liver function, i.e., it has an adaptive character. Given that the increase in CIC against the backdrop of administration of LMCC was rather small and LMCC was administered to animals *per os,* we may believe that it reflects the activation of the immune system.It is known that neutrophils phagocytize damaged cells of its own body, secrete antibacterial agents, and facilitate regeneration of damaged tissues (Pittman K. and Kubes P., 2013).To assess the ability of neutrophils to form phagocytes and to destroy foreign or autoimmune antigen complexes (CIC) are often used to test with nitro blue tetrazolium (NBT - test). NBT – test makes possible to evaluate the state of NADPH – oxidase activity of the neutrophils. NBT – test determines a spontaneous (initial, not activated) level of NADPH activity and (potentially possible) level of NADPH activity that was activated by zymosan (John M. Gansner and Nancy Berliner, 2016). It turned out that the animals with Cu-induced fibrosis had the NADPH activity baseline level that was by 57% above the control level (Fig. 3). Activation of neutrophils by zymosan resulted in an increase of the examined value by 2.8 times compared to the baseline in the control animals group and by 2.2 times in the group with fibrosis (Fig. 3, option 1, 2).

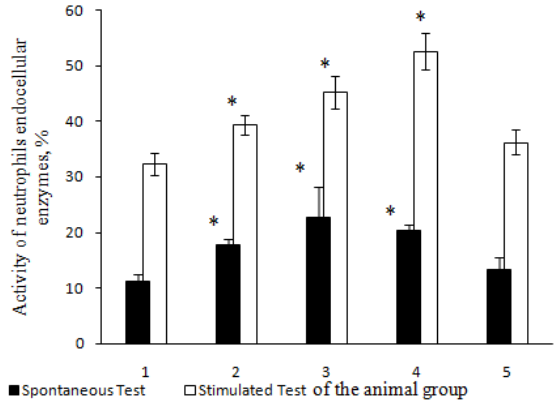


Figure 3. Spontaneous and stimulated level of neutrophils endocellular enzymes in the control animals (1), in animals with Cu-induced fibrosis (2), in animals with fibrosis, that was administrated with LMCC three times at the dose of 0.1 g/100 g of the body weight of the animals (3), the same at the dose of 0.05 g/100 (4), and at the dose of 0.01 (5). * – p < 0.05 compared to the intact control

Therefore, at early stages of formation of fibrosis an activation of neutrophils was observed that were able to form phagocytes and destroy antigenic complexes, the content of which increased in comparison with the control animals. At the same time, "potentials" of neutrophils in animals with fibrosis stayed high, though by 64% less than the intact control in comparison to the spontaneous level. In that case, if the animal with Cu-induced fibrosis was injected with LMCC at the dose of 0.1 g/100 g of the body weight of the animal, then the baseline level of neutrophil activity was higher in comparison to animals with fibrosis by 28 %. If those animals were administered with LMCC at lower doses (0.05 g and 0.01 g / 100 g of the body weight), then this index did not differ significantly from that in the animals with fibrosis, and at the dose of 0.01 g / 100 g of the body weight of the animal it corresponded to the intact control (Fig. 3, option 3-5). Therefore, there is dose dependence in activation of the baseline neutrophil activity in animals with fibrosis, which decreases with decreased doses of LMCC (Fig. 3).

The assessment of a potential level of activity of neutrophils in animals that were administered with LMCC against the backdrop of fibrosis revealed, that they were able to be activated 2.0; 2.6 and 2.7 times correspondingly at the doses of 0.1; 0.05; 0.01 g/100 g of the body weight of the animal (Fig. 3). Therefore, against the backdrop of fibrosis LMCC were able to activate neutrophils only at a large dose (0.1g / 100 g of the body weight) while their potential level of activity remained sufficiently high. It is known, that one of the major critical factors of pathogenesis for liver fibrosis, as well as for other pathological conditions is endogenous intoxication (Yurieva E.A. *et al*., 2015). It is believed that endogenous intoxication is associated with accumulation of metabolites from normal and abnormal metabolism in biological fluids of the body. There are three basic factors that induce endogenous intoxication - microbiological, biochemical and immunological (K. dePunderandL. Pruimboom, 2015). Cu-induced fibrosis may be related to biochemical and immunologic mechanisms of endogenous intoxication. Multiple studies demonstrated that the state of endogenous intoxication occurs in a variety of diseases and has no specific symptoms (V.N. TitovandV.V.

Krylin, 2010). A degree of development of endogenous intoxication can be an indication of a development stage of pathological process. The content of average molecular weight molecules in biological fluids is used as an indicator of toxicity of the internal environment (up to 5,000 D) and oligopeptides (V.I. Sidel'nikova *et al*., 2015). The determination of the AMWP content in the animals with Cu-induced fibrosis revealed that they were not different from the intact control (see the Table 1).

Introduction of LMCC to animals with fibrosis at doses of 0.1 and 0.05 g/100 g of the body weight, was accompanied with a slight increase of AMWP by 24; 48% in comparison with fibrosis and the intact control, while a dose of 0.01 g/100 g of body weight of the animal had no effect on this indicator. Therefore, the LMCC components increased the content of AMWP in the blood serum of the animals at the dose of 0.1 and 0.05 mg / 100 g of the body weight of the animal.

Table 1. Content of the average molecular weight peptides in experimental animals

	Experimental group of animals			
Indicator	Intact control	Cu – induced fibrosis	Cu – LMCC (0.1 g/ 100 g of the body weight of the animal)	Cu – LMCC (0.05 g/ 100 g of the body weight of the animal)	Cu – LMCC (0.01 g/ 100 g of the body weight of the animal)
AMWP	0.320 ± 0.02	0.331 ± 0.01	0.410 ± 0.03*	0.490 ± 0.09*	0.350 ± 0.07

p< 0.05 in comparison to the intact control;

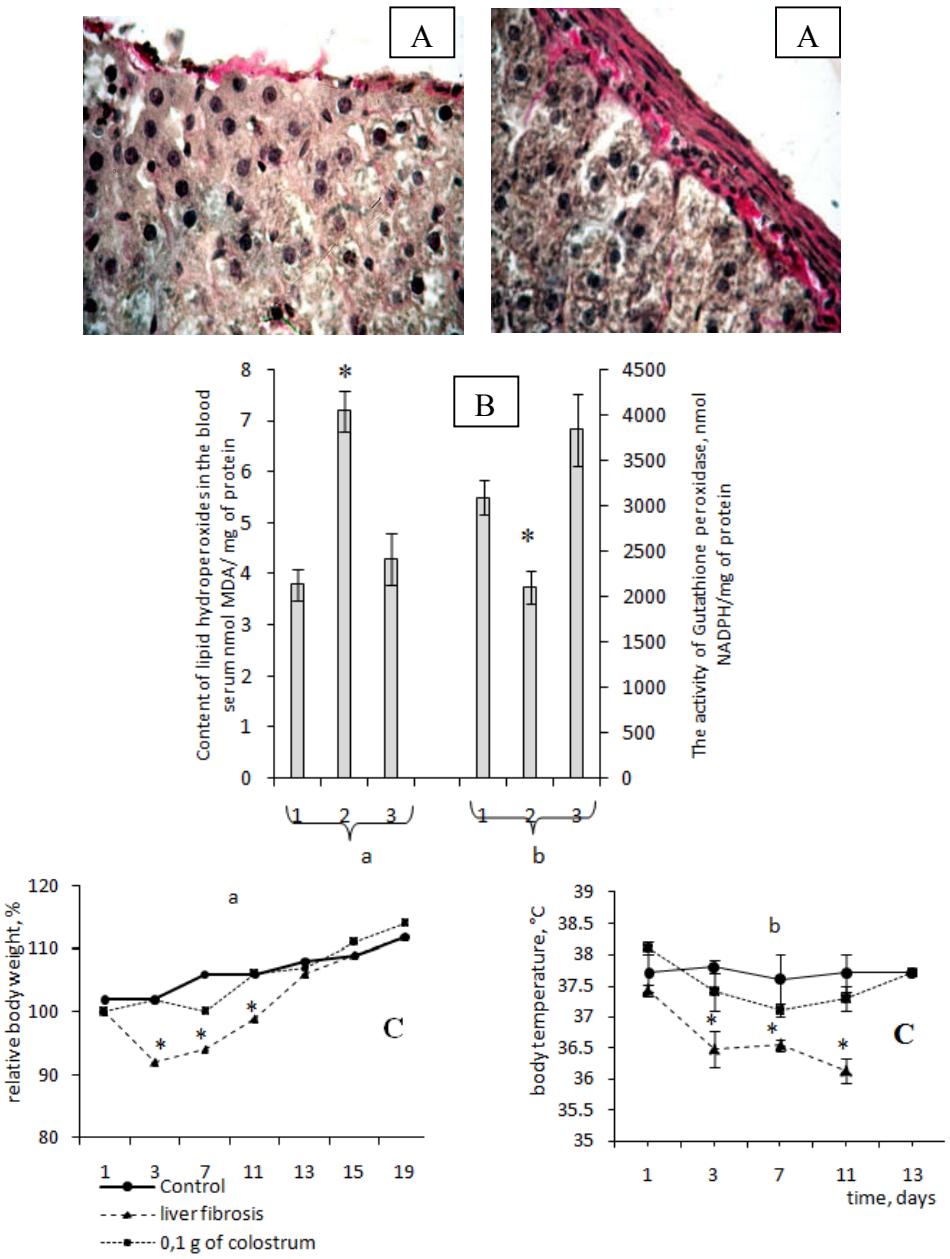


Fig. 4 Morphology of liver tissue (A) and of the intact liver (a) and rats with Cu-induced liver fibrosis (b), indices of the pro-/anti-oxidant system (B); content of lipid hydroperoxides in serum (a) and activity of glutathione peroxidase (b) in intact animals (1), animals with fibrosis (2), and animals with fibrosis treated with LMCC at a dose of 0.1 g/100 g of the body weight of the animal (3); some physiological data (C), body mass variation (a) and body temperature variation (b) in the intact animals (1), animals with fibrosis (2), and animals with fibrosis treated with LMCC at the dose of 0.1 g / 100 g of the animal body weight (3). p ≤ 0.05 in comparison to the intact control

Table 2. Some biochemical parameters those were determined in serum blood of the experimental animals

Experimental	Indicator			
group of animals	Cholesterol	Triacylglycerol	Creatinine	Albumen
Intact control	1,6 ± 0,2	0,77 ± 0,13	69,7 ± 15,1	32,3 ± 0,4
Cu – induced fibrosis	1,2 ± 0,4	0,79 ± 0,27	50,0 ± 3,9	29,3 ± 2,6
Cu – LMCC (0.01 g/ 100 g of the body weight of the animal)	1,5 ± 0,5	1,0 ± 0,48	93,0 ± 20,0	33,5 ± 4,0

It should be noted that AMWPs are not only an indicator of toxicity, but they may perform regulatory functions as well (Nikol'skayaV.A. *et al*., 2013 in Russian).

Some physiological parameters of the experimental animals and characteristic values of the pro-/anti-oxidant system in their blood serum

It is known that neutrophils play an important role in the inflammatory process. They actively migrate from the blood into a damaged area, as effectors and modulators of the acute phase of inflammation. A morphological analysis of the intact liver and the liver 24 hours after the last administration of copper sulfate showed that there was a large number of neutrophils and blood cells of other types in the liver capsule that was enlarged by many times in comparison to the control (Fig. 4 A). Therefore, after a 3-time sequential administration of copper sulfate to the experimental animals neutrophils rushed in the inflammatory focus. As it is known, the neutrophils in an inflammatory focus perform at least two important regulatory functions: microbicidal (by phagocytosis) and produce active forms of oxygen. Hyperproduction of active forms of oxygen by neutrophils (MaianskiN.A. *et al*., 2004) may lead to death of the cells, development of oxidative stress (MenshchikovaE.B. *et al*., 2006; Tiwari B.S. *et al*., 2002) and changes in the redux system of the cell. Determination of the content of serum lipid hydroperoxides in animals with Cu-induced liver fibrosis revealed that their content was increased by 90 % in comparison to the intact control (Fig. 4 B). At the same time, the activity of one of the antioxidant enzyme - glutathione peroxidase was reduced by 36% compared with the intact control (Fig. 4 B). In the case when the animals with fibrosis received LMCC at the dose of 0.1 mg / 100 g of the body weight, then the content of lipid hydroperoxides in the blood serum was not different from that of the intact control, and was by 40 % lower than with liver fibrosis (Fig. 4 B). An invert correlation was observed between the content of lipid hydroperoxides and the activity of glutathione peroxidase.

Thus, LMCC increases the activity of glutathione peroxidase in the animal blood serum with liver fibrosis by 82 % in comparison with fibrosis, and it was slightly higher (24 %) of the intact control (Fig. 4 B). Therefore, LMCC activated glutathione peroxidase and lowered the content of lipid hydroperoxides in the blood serum in animals with Cu-induced fibrosis. On the functional activity of liver fibrosis was evaluated by some biochemical parameters as well as cholesterol, triacylglycerol, albumin and creatinine. These parameters in the animals with Cu - induced fibrosis did not differ from control animals (see the Table 2). A manifestation of oxidative stress in animals with Cu-induced fibrosis was also accompanied with changes in physiological characteristics of the animals. Thus, if for the period of preparation for the experiment from the 1st to the 15th day, the weights of the three-month old animals increased by 12%, in animals with fibrosis itreduced by 10 g of the initial weight during the first 3 days (Fig. 4 C). However the body temperature of animals with liver fibrosis at that time was 1°C lower than that of the control animals (Fig. 4 C). In the cases when the animals with fibrosis were injected low molecular weight components of colostrum (LMCC) at 0.1 g/100 g of the body weight, their weight loss was much lower, and their body temperature did not differ from that of the control animals (Fig. 4 C). Therefore, LMCC eliminated the arrest of development and normalized the body temperature of animals with Cu-induced fibrosis.

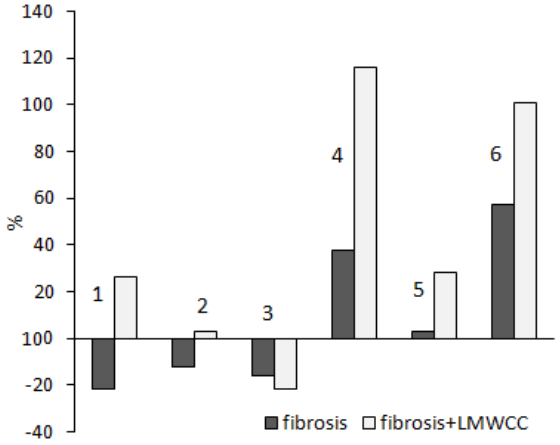


Fig. 5. Change of some immunological parameters in animals with Cu-induced fibrosis compared to control,which is taken as 100%, that was administered with LMCC at the dose of 0.1 g/100 g of the body weight of the animals

Onthex-axis: 1 – Phagocyticindex; 2- Phagocytic number; 3- Index of phagocytosis completion 4 – Circulating immune complexes; 5 – Average molecular weight peptides; 6 – Spontaneous level of neutrophils endocellular enzymes.

DISCUSSION

In experimental animals repeated administration of copper sulfate leads to the accumulation of high concentration of copper in the liver (A.I. Bozhkov *et al*., 2010). It is known that copper ions are essential to organism, they are components of more than 40 enzymes (Edward I. Solomon *et al*, 2014). Copper is bound and carried by specific proteins: ceruloplasmin, copper binding protein (COP), etc. (Tishchenko, KI *et al*., 2016). However, at high (non-physiological) concentrations the copper ions bind nonspecifically to a large number of proteins and inactivate these proteins (Mir-Jamal Hosseini *et al*., 2014). Thus, it was shown that after three sequential administration of copper sulfate 1 mg / 100 g of liver weight with 48 hour intervals copper became associated with specific COPs, mitochondria, proteins of cytosol, and modifies their functional characteristics (A.I.Bozhkov *et al*., 2014 in Russian). The results of the present study showed that the key enzymes glutathione cycle - glutathione was reduced activity by 36% after three consecutive copper sulfate administrations. At the

same time lipid hydroperoxide content in mitochondria increased by 50% compared to control (A.I. Bozhkov *et al*., 2016). This significant increase of products of free radical reactions in the liver has such consequences. First, it activates polymorphonuclear leucocytes and is a signal for the migration of phagocytic cells into the inflammatory focus, which can be seen in Fig. 4A. Second, the products of free radical reactions activate hepatic stellate cells, accompanied by a cascade of proinflammatory reactions (De Minicis S. *et al*., 2012). Third, the increase of free radicals and their reaction products induce necrotic and apoptotic changes and elimination of a part of cells and cell structures, and as a result, renovation the cell structures in the liver (H. Cichoż-LachandA. Michalak, 2014).
Neutrophils are the most numerous population of phagocytes in human organism (AttilaMócsai, 2013). Neutrophils play an important role in the "supervision" of the homeostasis of the organism, and are the expression of S.O. Iseri element of the first line of defense. It is known that neutrophils use two mechanisms for the implementation of the protective effect: the formation of phagosomes and "oxygen burst" (Christine C. *et al*., 2016). "Oxygen burst" provides neutralization of infectious agents, destabilize the membrane of phagocytic cells, participating in the regulation of biosynthesis and destroys damaged liver tissue, helping to remove the damaged cell structures (C. J. Harbor*et al*., 2016). As a result of "oxygen burst" in neutrophils the free-radical reactions products in serum is increased to 90% compared to control level (Fig. 4).

For neutrophils characterized by an extremely high intensity of redox processes. High speed metabolic adjustment stimulated neutrophils and gave rise to the phenomenon of comparison with the explosion, which was called "metabolic" oxygen "or" oxidative "burst (C. J. Harbor*et al*., 2016). It is established that the main enzyme producing free radicals at oxidative burst is NADPH oxidase (Christine C. *et al*., 2016). This enzyme is located in the phagolysosomes and converts $O_2O_2^-$ (NADPH + $2O_2 \leftrightarrow NADP^+ + 2O_2- + H^+$). Determination of NADPH oxidase activity in HCT-test showed that it significantly increased in animals with Cu-induced fibrosis (Fig. 3). It should be noted that in a case ofCu-induced fibrosis the increase in products of free radical reactions was due to inhibition of antioxidant enzymes by copper ions, including glutathione peroxidase and due to the oxygen burst in neutrophils (Fig. 3, 4). This may explain the fact that in the animals with Cu-induced fibrosis in hepatocytes and in blood serum the hydroperoxide content increased, correspondingly, by 30 - 40%, and 90-100%. Along with the oxidative damage of cells the activation of autoimmune process was registered, as evidenced by the significant increase of CEC in serum (Fig. 2B). These dynamic changes in the liver and in the whole organism of animals' thrice-received copper sulfate are adaptive at the initial stages of Cu-induced fibrosis (24 hours after the last administration of copper sulfate). In favor of this suggestion evidence the data on conservation of liver functional activity at the control level in this period (Table. 2).

In favor of the adaptive nature of changes detected in cells in our experiments are also evidences or the hormesis effect to high concentrations of copper. Thus, after three consecutive injections of copper sulfate, animal acquired resistance to higher doses of copper sulfate (A.I. Bozhkov *et al*., 2010)
However, along with oxidative stress and activation of cellular immunity some of liver functions change. The body mass and the whole metabolism decrease (the body temperature significantly decreases on 1^oC). Animals with Cu-induced fibrosis had normal body temperature if they received LMCC, and the body mass wasn't differed on control. Glutathione peroxidase activity increased by 82% compared to fibrosis and even exceeded that of control animals. The content of lipid hydroperoxide in blood serum recovered to control level (Fig. 4B). The activity of neutrophils in animals treated with LMCC on against the backdrop of fibrosis, even more increased. Content of CIC and phagocytic indexwere increasedin the animals (Fig. 5).

In addition, content of average molecular weight peptides was increased in animals with fibrosis that received LMCC.These results suggest that the LMCC has a marked immunotropic activityand elimination of oxidative stress and activation of cellular immunity correlated with normalization of the functional characteristics of animals. Therefore, there is no direct correlation between indications of activity of the pro-oxidant system and activation of the cellular component of the immunity. It is possible to state that different variants of relationships from a functional dependence to a complete or a partial autonomy of response reactions to actions of various factors are characteristic for the system of pro-oxidants and the cellular component of the immunity system. Cellular immunity can be activated both an increase in products of free radical reactions and LMCC components. The obtained results allow us to present the following explanations. Low molecular weight components of colostrum, consisting of low molecular weight peptides, amino acids, transfer-factor, and other cytokines (BrianA. McGrath*et al*, 2016), are able to demonstrate a polyfunctional action. Some of those components possess an antitoxic property, activate glutathione peroxidase, and as the result, lower the content of resultant products of free-radical reactions. Other components possess immunogenicity and have a direct effect on the immune system.

Therefore, LMCC had both a direct and indirect effect on the pro-antioxidant and the immune system. It is important that establishment of new patterns in those systems as the result leads to alteration of all the reductive-oxidative systems of the body that provide regulation of activity of those two and other body systems.

In conclusion, we would like to note:

- Activation of Cu-induced fibrosis begins with a manifestation of oxidative stress and activation of the cellular component of the immune system;
- Already in the early stages of development of liver fibrosis the animals fell behind in their growth rate, their body temperature and performance decreased in 1 $^{\circ}C$;
- Oral administration of low molecular weight components of colostrum at the early stages of fibrosis development eliminated the manifestations of oxidative stress, activated the activity of glutathione peroxidase and the immune response, restored the growth rate, body temperature, and performance ability.

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