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Research Article

Mafusol and its combination with REXOD as correctors of reduced blood circulation in the skin associated with normoglycemia or diabetes mellitus complicated by exogenous hypercholesterolemia

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Abstract

Introduction: The search for and development of new highly active medications and their combinations of the appropriate direction of action remains an urgent problem due to the complications of diabetes mellitus, especially burdened with atherosclerosis, including skin and vascular lesions.

Materials and methods: The acute toxicity, histoprotective and dermatoprotective effects of mafusol, rexod, alprostadil and their combinations were studied in male rats with normoglycemia and alloxan diabetes complicated by exogenous hypercholesterolemia.

Results: The combination of mafusol with rexod is less toxic than mafusol. In arteriovenous insufficiency of the tail, ischemia of the skin fold and skin flap, mafusol (6.25, 12.5 and 25.0 mg/kg in terms of fumarate), rexod (0.01 and 0.02 mg/kg) and especially their combination (6.25 and 0.01 mg/kg) have significant histoprotective, dermatoprotective, hypoglycemic and lipid-lowering effects, both in normoglycemia and alloxan diabetes complicated by exogenous hypercholesterolemia. Alprostadil (10 mg/kg) and especially its combination with mafusol (6.25 mg/kg) have a dermatoprotective effect.

Discussion: Rexod reduces the acute toxicity of mafusol. The dermatoprotective effect of mafusol, rexod and, to a greater extent, their combination may be associated with increased microhemocirculation, antihypoxic properties and activation of energy processes in the skin, normalization of carbohydrate and lipid metabolism in alloxan diabetes, complicated by exogenous hypercholesterolemia, increased reserve capacity of the antioxidant system, and possibly

with the ability of mafusol and rexod to reduce blood viscosity and improve rheological properties of the blood. The combination of mafusol with alprostadil increases the dermatoprotective activity of the latter.

Conclusion: Combinations of mafusol with rexod and alprostadil can be recommended for clinical study as dermatoprotective agents for treating traumatic injuries and diabetes mellitus complicated by atherosclerosis.

Keywords

mafusol, rexod, alprostadil and their combinations, skin, reduced blood circulation, alloxan diabetes, exogenous hypercholesterolemia.

Introduction

It is known that microcirculatory disorders accompany many diseases, in particular diabetes mellitus (DM), and entail changes in the regulation of central and regional blood circulation, shifts in the viscosity and volume of blood circulation, the formation of defects in the blood vessel walls, etc. In this regard, the chronic complications of DM, accompanied by atherosclerosis, micro- and macroangiopathies, are worth noticing (Volmer-Thole and Lobmann 2016; Lim et al. 2017; Zhang et al. 2017; Buckley et al. 2018; Starostina 2018; Bensman et al. 2019; Khramilin and Demidova 2020; Shinkin et al. 2020).

One of the promising direction in the pharmacological treatment of microcirculatory disorders in various organs and tissues, including the skin, particularly in DM and atherosclerotic vascular lesions, is both the separate and combined use of antioxidants and antihypoxants (Shakhmardanova et al. 2016; Seletskaya and Galenko-Yaroshevsky 2017; Galenko-Yaroshevsky et al. 2018, 2018a).

Taking into account the above, for our study we chose the drugs mafusol and rexod. The first one contains fumaric acid, which is part of the Krebs cycle and ensures the maintenance of energy processes in tissues at the appropriate level (Strakhov 2016; Sukhomlin et al. 2016; Maevsky and Grishina 2017; Sukhomlin et al. 2017); the second one, which is a recombinant human superoxide dismutase (SOD), is the first line enzyme of defense against free radicals and also has a pronounced anti-inflammatory, anti-hypoxic, cytotropic and membrane tropic activities (Shakhmardanova et al. 2016; Jiang et al. 2017; Biosa et al. 2018; Nguyen et al. 2018; Saroyan et al. 2018; Shafranova et al. 2018; Srivastava et al. 2018; Wang et al. 2018; Yoon et al. 2018; Shi et al. 2019; Leontev et al. 2020).

The aim of the study was to increase the viability of the skin with reduced blood circulation against the background of normoglycemia or experimental diabetes mellitus, complicated by exogenous hypercholesterolemia, using the combination of mafusol and rexod.

Materials and methods

The experiments were performed in 750 white outbred male rats, including 16 newborn baby rats, weighing 170–245 g and 5–6 g, respectively. The requirements of the Law of the Russian Federation "On the Prevention of Cruelty to Animals " of 24.06.1998, the Rules of Laboratory Practice in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), World Medical Association Declaration of Helsinki (2001), the European Society Directive (86/609 EC), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1997), as well as the Rules of Laboratory Practice adopted in the Russian Federation (order of the Ministry of Health of the Russian Federation No. 708 of 29.08.2010) were taken into account. All the experiments were approved by the Ethics Committee of Kuban State Medical University (Protocol No. 5 of 23.11.2017).

The acute toxicity of mafusol, rexod and their combinations was determined in rats with intraperitoneal administration. The average lethal doses causing the death of 50% of animals (LD_{50}) were determined (Arzamastsev et al. 2000).

The study of the histo- and dermatoprotective effects of mafusol, rexod and their combinations in reduced blood circulation against the background of normoglycemia was carried out in rats' tails, using the methods described by Seletskaya and Galenko-Yaroshevsky (2017).

The study of the effect of alprostadil and its combination with mafusol and rexod on the viability of the skin flap of the anterior abdominal wall in partial arteriovenous insufficiency and normoglycemia was conducted in rats, using the method described by Galenko-Yaroshevsky et al. (2018).

The study of the effect of mafusol with rexod combination on the viability of the skin in partial arteriovenous insufficiency against the background of carbohydrate and lipid metabolism disorders was performed in rats according to the method described by Seletskaya and Galenko-Yaroshevsky (2017) and Galenko-Yaroshevsky et al. (2018). Alloxan diabetes (AD) was simulated by intraperitoneal administration of alloxan to the rats at a dose of 135 mg/kg. Two weeks after the AD simulation, cholesterol was introduced intragastrically through a tube (40 mg/kg in 0.5 ml of vegetable oil) for 14 days. To increase peroxide stress, ergocalciferol was added to the emulsion (at the dose of 12500 U/kg). At the same time, the experimental group of the animals was administered

intravenously with mafusol (at the dose of 6.25 mg/kg) in combination with rexod (0.01 mg/kg), whereas the control group was administered with saline solution in the equivalent volume for 14 days. The blood levels of glucose, total cholesterol, triglycerides, beta-lipoproteins and high-density lipoproteins (HDL) were determined in an automatic biochemical analyzer KONELAB-30 (Thermo Fisher Scientific Corporation, USA).

The study of the effect of a new injectable form of Rexod® on microhemocirculation (MHC) in the rat skin was performed by laser Doppler fluometry method, described by Chuyan et al. (2017) and Galenko-Yaroshevsky et al. (2018). Following non-oscillatory data of basal blood flow were recorded: microcirculation indicators, standard deviations, and coefficients of variation. In addition, the oscillatory amplitudes of blood flow in different frequency ranges were determined by wavelet analysis, reflecting endothelial, neurogenic, myogenic, respiratory and pulse processes.

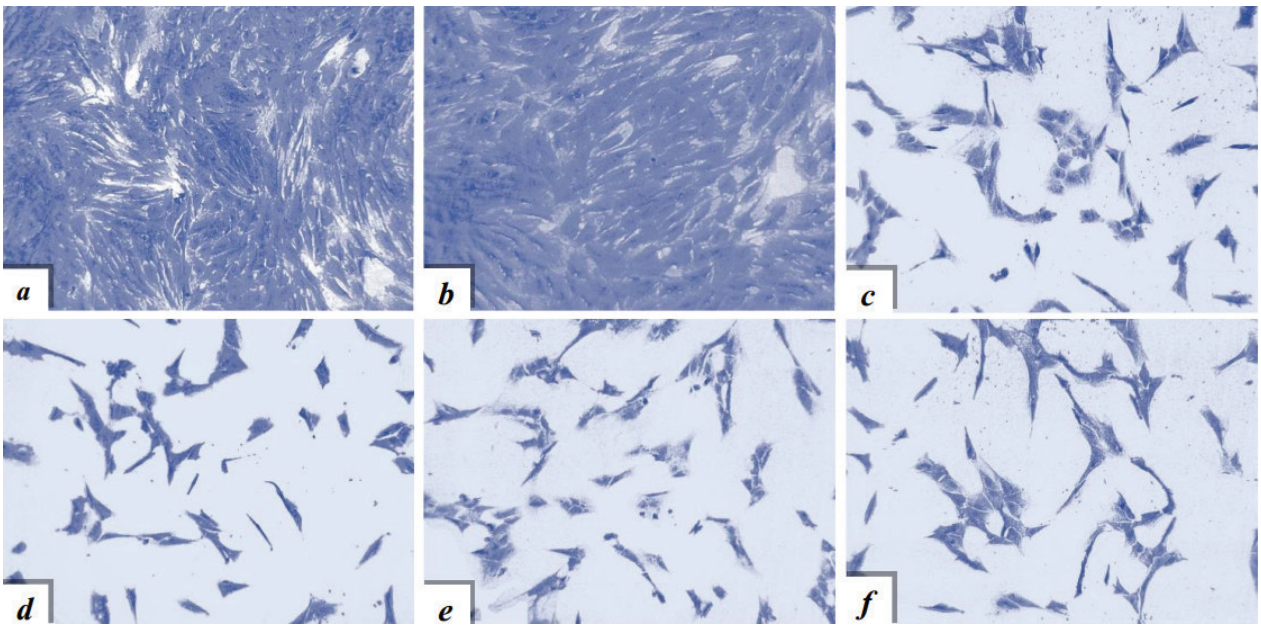
The study of the effect of mafusol, rexod and their combination on the metabolic activity of the osteoblasts (parietal bone) and fibroblasts (skin) of the newborn rats was carried out according to the method described by Seletskaya and Galenko-Yaroshevsky (2017).

Hypoxia was simulated by adding NaCN and 2,4-dinitrophenol (DNP) to the Costar plate wells to the final concentration of 0.2 and 0.5 mM, respectively, followed by incubation for 24 hours (Seletskaya and Galenko-Yaroshevsky 2017) (photomicrography).

The metabolic activity of osteoblasts and fibroblasts in the presence of mafusol, rexod, and their combinations was evaluated using the MTT assay, which is based on the ability of dehydrogenases to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into insoluble purple formazan crystals (Seletskaya and Galenko-Yaroshevsky 2017).

The study of the effect of mafusol, rexod and their combination on the indicators of energy supply, antioxidant defense network (ADN), and the intensity of the necrotic process in the ischemia of the dorsal skin fold of rats against the background of normoglycemia. The material for biochemical studies was taken on the 4th day from the middle part of the skin fold. The content of adenylic (ADP) and pyridinic (ATP) nucleotides, creatine phosphate (CP), cytochrome C, lactate, pyruvate, malondialdehyde (MDA), as well as the enzymatic activity of the succinate and NADH-ubiquinone-reductase systems, glycolytic activity, SOD and catalase activities were determined in non-nuclear skin homogenates using the methods described by Seletskaya and Galenko-Yaroshevsky (2017). The activity of creatine phosphokinase (CPK) was studied using a LaRoche test kit; the activity of glutathione peroxidase (GP) and the rate of superoxide anion generation (O_2^-) – by the Chen et al. method (2000); the activity of lactate dehydrogenase (LDH) was determined using a Diakhim test kit by the reaction with 2,4-dinitrophenylhydralazine, and α-ketoglutarate dehydrogenase – by the Lai and Cooper method (1986). The levels of alanine and aspartate aminotransferases (ALT and AST) were studied using Bio-La-Test (Lachema) kits. The cytolysis index was calculated as the CPK/AST ratio. The coenzyme Q_{10} level was determined according to the method described by Seletskaya and Galenko-Yaroshevsky (2017).

Statistical processing of the study results collected in an alternative form was carried out according to Arzamastsev et al. (2000), and in the graded form – using licensed software Microsoft Office professional plus 2013 and STATISTICA–8.0. The significance of the differences



Photomicrography. The effect of NaCN and DNP on the proliferative activity of osteoblasts and fibroblasts of the newborn rats. **Note:** ***a*** and ***b*** – primary monolayer culture of osteoblasts and fibroblasts; ***c*** and ***d*** – osteoblasts in the presence of NaCN and DNP (0.2 and 0.5 mM); ***e*** and ***f*** – fibroblasts in the presence of NaCN and DNP (0.2 and 0.5 mM). Trypan blue stain. Magnification ×100.

in the indicators of the control and experimental groups of the animals was determined using the Mann-Whitney U-test, after Shapiro-Wilk normality test. The differences between the indicators of the control and experimental groups were considered statistically significant at $p \leq 0.05$.

Results

Acute toxicity, histoprotective and dermatoprotective effects of mafusol, rexod, alprostadil and their combinations in reduced blood circulation in rats

Acute toxicity of mafusol, rexod and their combinations. The acute intraperitoneal median lethal dose (LD_{50}) of mafusol (in terms of sodium fumarate) in rats was 637.5 mg/kg. The administration of rexod at the dose of 1 mg/kg (400000 U/kg) did not cause any signs of poisoning both in the short-term (3 hours) and in the long-term (within 14 days) periods of observation. The LD_{50} of mafusol injected with rexod (1 mg/kg intraperitoneally) was 725.3 mg/kg (Table 1).

Table 1. Acute Toxicity of Mafusol and Its Combination with Rexod in Intraperitoneal Administration to Rats

Medication	Acute toxicity indicators		
	LD_{50}, mg/kg	tgα[1]	PR[2]
Mafusol	637.5 (630.7÷643.9)	1.24 (1.02÷1.45)	1.14 [1.03] (1.11÷1.17)
Mafusol + rexod (1 mg/kg)	725.3 (707.6÷743.0)	1.12 (0.99÷1.25)	

Note: in round brackets – confidence intervals at p=0.05, in square brackets – f_{PR} factor; [1] – regression slope reflecting the dose-effect relationship; [2] – differences between the LD_{50} values.

Graphical determination of the tangents of the angles α (tgα) formed by the regression lines and reflecting the dose-effect relationship (deaths) of the compared mafusol and the combination of mafusol with rexod and the abscissa axes showed that tgα for mafusol was equal to 1.24, and for the combination – 1.12, while the difference between them was statistically insignificant (Table 1). That is, the regression lines are almost parallel, which gives the possibility to calculate the ratio (PR) of values of their activity or acute toxicity. Based on the above statement, we found that the PR of LD_{50} of the combination of mafusol with rexod to LD_{50} of mafusol was 1.14, while the f_{PR} factor was 1.03. These data eventually allowed us to calculate the confidence intervals for PR and present the indicators in the following form: PR = 1.14 (1.11÷1.17) at p = 0.05 (Table 1). Since the ratio of the compared parameters (PR = 1.14) exceeds the value of f_{PR} (1.03), the difference in toxicity of mafusol and the combination of mafusol with rexod can be considered as statistically significant (at p = 0.05). Thus, the combination of mafusol with rexod in intraperitoneal administration to rats shows 1.14-time less acute toxicity than mafusol only.

In the experiments on the rat tails (*with simultaneous ligation of all vessels with the surrounding tissues*, *except for the dorsal arteries and veins*), mafusol (0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50 mg/kg) and rexod (0.01, 0.02, and 0.04 mg/kg) with intravenous and intraperitoneal administrations have a histoprotective effect; the most effective doses of the first one are 6.25 and 12.5 mg/kg, and of the second one – 0.01 and 0.02 mg/kg; on the 7th day of the study, the surviving part of the tails, when compared to the control, was 58.3 and 99.7, 28.1 and 32.3%, respectively. The combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) causes a significant increase in the histoprotective effects of these medications; on the 7th day of the study, the surviving part of the tails compared to the control was 99.5% (Table 2).

Table 2. The Effect of the Combination of Mafusol and Rexod on the Survival of Rat Tail Tissues in Conditions of Arteriovenous insufficiency (M ± m, n=10)

Medication, group	Dose, mg/kg	Necrosis length, %	
		3rd day	7th day
Normal saline, Control [1]	–	55.7±6.9 (35.6÷71.8)	88.4±2.8 (82.0÷94.8)
Mafusol+ rexod [2]	6.25 0.01	0.0±0.0 (0.0÷0.0) 55.7/100.0 p_{2-1}<0.001	0.4±0.4 (-0.5÷1.2) 88.0/99.5 p_{2-1}<0.001

Note: in round brackets – confidence intervals at p=0.05, in square brackets – animal group number; in the numerator – difference in the length (as %) of the necrotized part of the tails of the control and experimental groups of animals; in the denominator – – the surviving part (as %) of the tails (when comparing the necrotized parts) of the control and experimental groups.

Single and multiple (once a day for 7 days) intravenous administration of mafusol (6.25 mg/kg), rexod (0.01 mg/kg) and especially their combination (6.25 and 0.01 mg/kg, respectively) to rats with simulated dorsal skin fold and skin flap at the anterior abdominal wall with arteriovenous insufficiency has a pronounced dermatoprotective effect. Thus, on the 7th day of the experiment with the skin fold with a single administration of mafusol, rexod and their combination at the studied doses, the surviving part of the skin was 42.1%, 37.2%, and 57.1%, whereas with multiple administration of these medications and their combination – 64.4%, 43.5%, and 84.5%, respectively (Fig. 1).

It should be noted that the dose escalation of mafusol (12.5 mg/kg), rexod (0.02 mg/kg) and their combination (12.5 and 0.02 mg/kg) with repeated administration did not cause a statistically significant increase in their dermatoprotective effect compared to the previous doses.

In the experiments with of the anterior abdominal wall on the 10th day of the study, with a single and multiple administrations of mafusol, rexod and their combination at the doses of 6.25, 0.01 and 6.25 + 0.01 mg/kg, the surviving part of the skin was 23.5 and 23.3, 24.6 and 23.0, 63.6 and 53.7%, respectively (Figs 2, 3).

It is noteworthy that whereas in the skin fold, the dermatoprotective effect of the medications and their combination is more significant with the 7-times administration, in the skin flap, there were no differences in the skin survival rate between a single and multiple administration of these substances, which may be due to differences in the simulation of ischemic disorders in the skin, features of blood supply

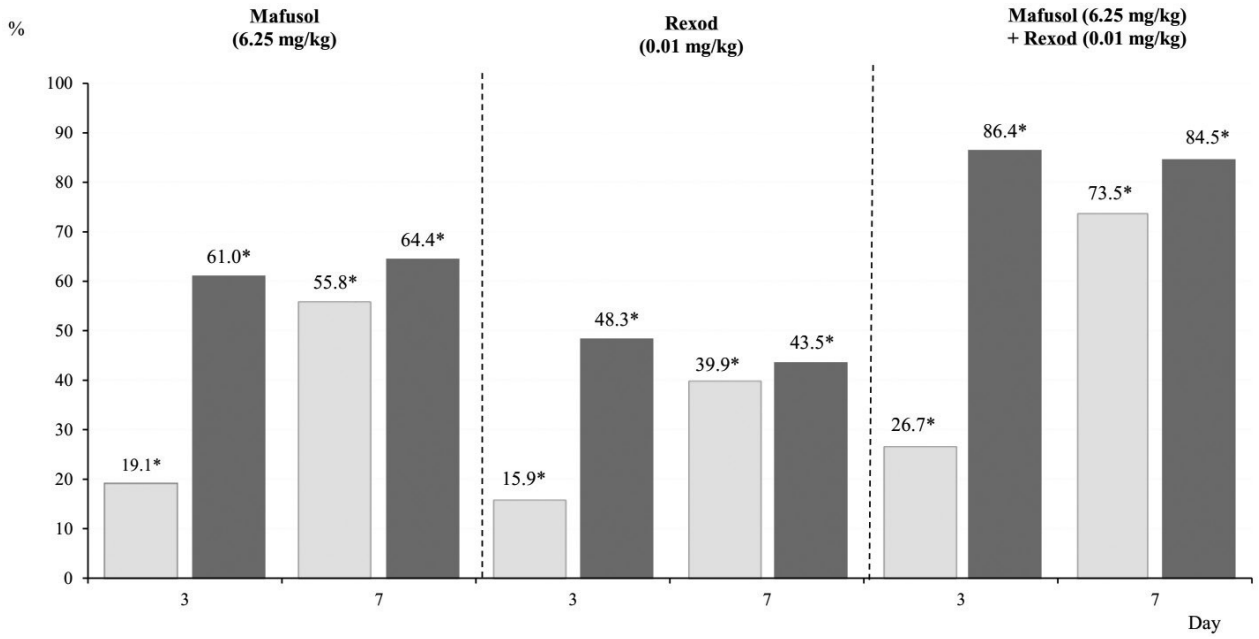


Figure 1. The effect of mafusol, rexod and their combination (7-time intravenous administration) on the survival of the dorsal skin fold in rats. **Note**: light gray bar charts – difference in the necrosis lengths of the ischemic skin folds of the control and experimental groups of animals on the 3rd and 7th day, dark gray bar charts – difference in the length of the surviving part on the 3rd and 7th day; * – differences are statistically significant compared to the control.

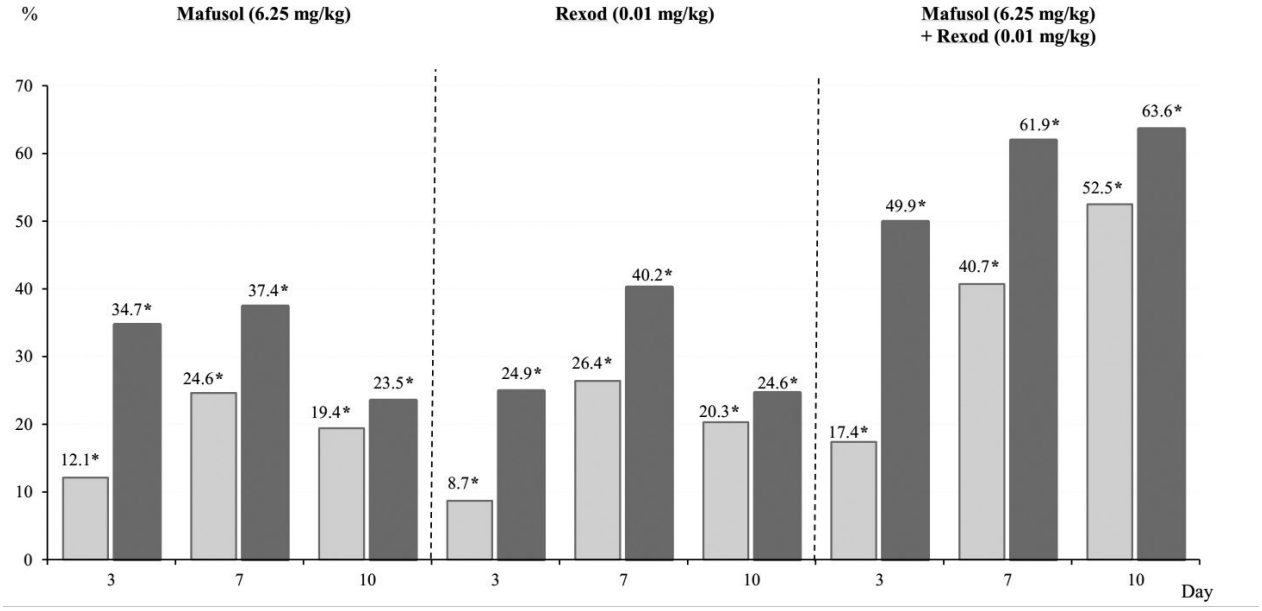


Figure 2. The effect of mafusol, rexod and their combination (single intravenous administration) on the survival of the skin flap of the anterior abdominal wall with its partial arteriovenous insufficiency in rats. **Note**: light gray bar charts – difference in the necrosis areas of the skin flaps of the anterior abdominal wall of the control and experimental groups of animals on the 3rd, 7th and 10th day, dark gray bar charts – difference in the areas of the surviving parts on the 3rd, 7th and 10th day; * – the differences are statistically significant compared to the control.

to the skin in the back and abdomen, as well as the changing targeted delivery of the studied drugs and their combination.

Alprostadil (10 mg/kg) and especially its combination with mafusol (6.25 mg/kg) with a single intravenous administration to rats have a dermatoprotective effect, increasing the survival rate of the skin flap of the anterior abdominal wall with partial arteriovenous insufficiency on the 10th day of the experiment (by 41.9% and 62.7%, respectively). The combination of alprostadil with rexod (10 and 0.01 mg/kg, respectively) under the same experimental conditions showed no dermatoprotective effect, which is accorded with the data of Seletskaya and Galenko-Yaroshevsky (2017).

Study of the effect of the combination of mafusol with rexod (6.25 and 0.01 mg/kg, respectively) on the survival of the skin flap of the anterior abdominal wall with its partial arteriovenous insufficiency against the back-

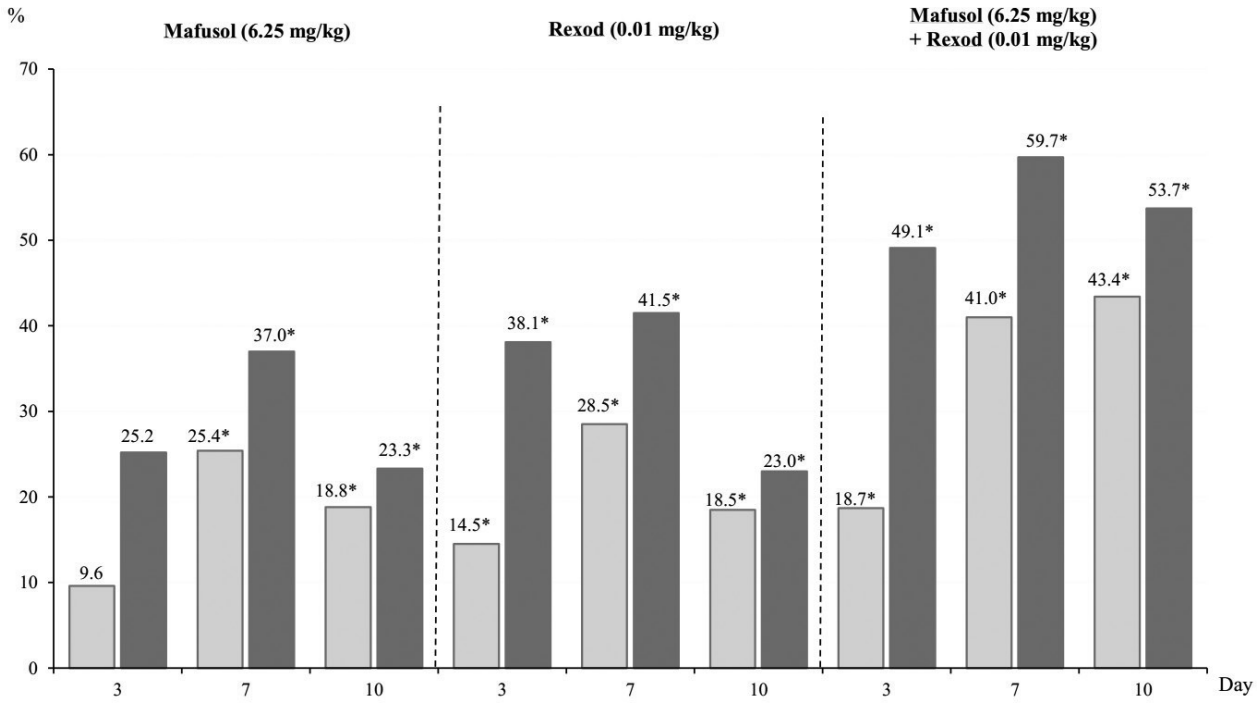


Figure 3. The effect of mafusol, rexod and their combination (7-time intravenous administration) on the survival of the skin flap of the anterior abdominal wall with its partial arteriovenous insufficiency in rats. **Note**: light gray bar charts – difference in the necrosis areas of the anterior abdominal wall of the control and experimental groups of animals on the 3rd, 7th and 10th day, dark gray bar charts – difference in the areass of the surviving part on the 3rd, 7th and 10th day; * – the differences are statistically significant compared to the control.

ground of hyperglycemia caused by AD and complicated by exogenous hypercholesterolemia (EHC) in rats, revealed that a multiple (once per day for 14 days) intravenous administration of the combination causes an increase in the survival rate by 30.2% on the 10th day after skin flap simulation compared to the control (Table 3).

Table 3. The effect of the Combination of Mafusol with Rexod on the Survival of the Skin Flap of the Rat Anterior Abdominal Wall with Its Partial Arteriovenous Insufficiency Against the Background of Carbohydrate and Lipid Metabolism Disorders (M±m, n = 13)

Medication combinations, doses, experimental conditions	Necrosis area of the skin flap, %		
	3rd day	7th day	10th day
Normal saline, normoglycemia [1]	28.8±1.3 (25.9÷31.7)	51.9±1.5 (48.7÷55.1)	68.0±0.9 (66.0÷70.0)
Mafusol, 6.25 mg/kg + Rexod, 0.01 mg/kg, normoglycemia [2]	18.1±2.1 (13.6÷22.6) 10.7/37.2 p_{2-1}<0.02	42.2±4.6 (32.2÷52.2) 9.7/18.7 p_{2-1} >0.05	47.0±3.7 (38.9÷55.1) 21.0/30.9 p_{2-1}<0.001
Normal saline, Alloxan diabetes + exogenous hypercholesterolemia [3]	33.3±1.4 (30.3÷36.3) p_{3-1}<0.05 p_{3-2}<0.001	63.2±1.2 (60.5÷65.9) p_{3-1}<0.001 p_{3-2}<0.001	82.8±1.0 (80.6÷85.0) p_{3-1}<0.001 p_{3-2}<0.001
Mafusol, 6.25 mg/kg + Rexod, 0.01 mg/kg, Alloxan diabetes + exogenous hypercholesterolemia [4]	19.0±1.5 (15.8÷22.2) 14.3/42.9 p_{4-1}<0.001 p_{4-2}>0.05 p_{4-3}<0.001	51.7±1.6 (48.3÷55.1) 11.5/18.2 p_{4-1}>0.05 p_{4-2}>0.05 p_{4-3}<0.001	57.8±1.6 (54.2÷61.4) 25.0/30.2 p_{4-1}<0.001 p_{4-2}<0.02 p_{4-3}<0.001

Note. In round brackets – confidence intervals at p = 0.05, in square brackets – animal group number; in the numerator – difference in the necrosis area (as %) of the control and experimental groups, in the denominator – the surviving part (as %) of the cells (when comparing the necrotized parts) of the control and experimental groups.

The study of the carbohydrate and lipid metabolism when using the combination of mafusol with rexod (*6.25 and 0.01 mg/kg*, *respectively*, *intravenously*, *once a day for 14 days*) *against the background of both normoglycemia and AD*, *complicated by EHC*, showed that the combination had a significant hypoglycemic effect, leading to a decrease in total cholesterol, triglycerides and an increase in HDL (in the blood); having no significant effect on the level of beta-lipoproteins in normoglycemia and contributing to a decrease in their level against the background of hyperglycemia (Fig. 4).

To study the mechanism of the dermatoprotective activity of mafusol, rexod and their combination to get a better understanding of it, we used not only the data we obtained on the pharmacodynamics of these drugs, but also took into account the literature data about their therapeutic properties. So, given the fact that mafusol shows the most pronounced detoxification effect when used with medications improving the rheological properties of the blood and MHC in tissues (Sukhomlin et al. 2016), it was important to investigate the effect of rexod on MHC in the skin, since ad hoc studies in this direction have not been conducted before. According to Boyarinov (2016), rexod increases the blood rheology due to inhibiting platelet aggregation caused by excessive accumulation of dioxide O_2^-.

Taking into account the above, we first investigated ***the effect of rexod (a new injectable dosage form) on MHC*** in the rat skin using laser Doppler fluometry. It was found that rexod (0.02 mg/kg intraperitoneally) causes activation of MHC in the skin, characterized by an increase in

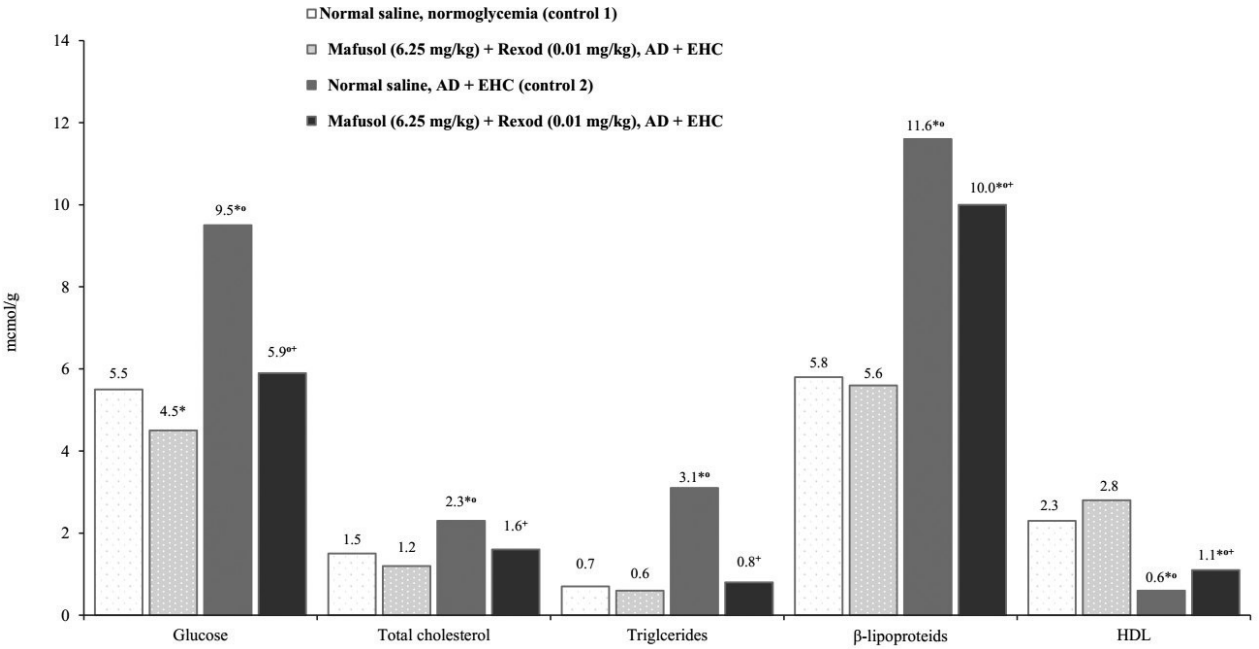


Figure 4. The effect of the combination of mafusol with rexod on the glucose and blood lipid levels in the rats against the background of normoglycemia or AD complicated by EHC. **Note**: the differences are statistically significant: * – compared to control 1; ° – compared to the combination of mafusol (6.25 mg/kg) + rexod (0.01 mg/kg), normoglycemia; + – compared to control 2.

both endothelium-dependent and endothelium-independent vasodilation, an increased metabolic activity of the vascular endothelium, a decreased adrenergic regulation of vasomotor reactions, a decreased peripheral vascular resistance, an increased blood flow to the microvasculature and an improved postcapillary outflow.

In vitro studies of mafusol (1, 5 and 10 mg/ml), rexod (0.0001, 0.0005 and 0.001 mg/ml) and their combinations (1 and 0.0001, 5 and 0.0005, 10 and 0.001 mg/ml) in cultured osteo- and fibroblasts under normoxia and histotoxic hypoxia caused by NaCN (0.2 mM), an inhibitor of tissue respiration enzymes, primarily mitochondrial cytochrome C oxidase, inducing loss of the ability of tissues to synthesize adenosine triphosphate and absorb oxygen (Seletskaya and Galenko-Yaroshevsky 2017), showed that mafusol and rexod had no significant effect on the cell culture. The exception was the combination of mafusol (5 mg/ml) with rexod (0.0005 mg/ml), which significantly increased the proliferative activity of osteoblasts.

In histotoxic hypoxia caused by (*DNP*) (*0.5 mM*), a decoupler of oxidative phosphorylation impairing energy production (macroergic compounds) and creating a deficiency of macroergic compounds in cells (Seletskaya and Galenko-Yaroshevsky 2017), mafusol (1 mg/ml), rexod (0.0001 and 0.0005 mg/ml) and their combinations (in the noted dilutions) significantly increase the viability of cultured osteoblasts and fibroblasts, but an increase in the concentrations of the studied substances (5–10 and 0.001 mg/ml, respectively) leads to a decrease in their activity, mainly towards osteoblasts.

Study of the effect of medications and their combination on energy processes in the experiments on the ischemic dorsal skin fold in the rats revealed that mafusol (12.5 mg/kg, intravenously) significantly increases the concentration of macroergic compounds necessary for activating and maintaining energy-dependent metabolic processes at an adequate level, and has the antihypoxic effect, which is confirmed by a decrease in the lactate concentration.

Rexod (0.02 mg/kg, intraperitoneally) exhibits an antihypoxic effect, which is evidenced by a decrease in the lactate concentration, which, unlike mafusol, is mainly due to an increase in the level of intermediates of redox reactions and energy metabolism.

The combination of mafusol (6.25 mg/kg, intravenously) with rexod (0.01 mg/kg, intraperitoneally) has a synergistic effect, which is manifested both in an increase in the intermediates of energy metabolism (pyridine-dependent coenzymes), and in an increase in the concentration of end products in the form of macroergic compounds. This may also indicate a possible increase in the proportion of aerobic processes relative to anaerobic processes as a result of a decrease in the severity of tissue hypoxia (Figs 5–9).

The study of the effect of the medications and their combination on the antioxidant defense network (*ADN*) revealed that mafusol (12.5 mg/kg, intravenously) has a positive effect on it, manifested by an increase in the activity of SOD, GP and catalase, which are involved in the neutralization of reactive oxygen species (ROS). Mafusol has no positive effect on peroxidation products, such as MDA, and on factors contributing to the additional formation of ROS, determined by the rate of O_2^- generation.

Rexod (0.02 mg/kg, intraperitoneally) causes an increase in the activity of endogenous SOD and GP, which is important for neutralizing aggressive compounds of ROS, but the activity of catalase remains unchanged. At the same time, the drug reduces the concentration of MDA, which,

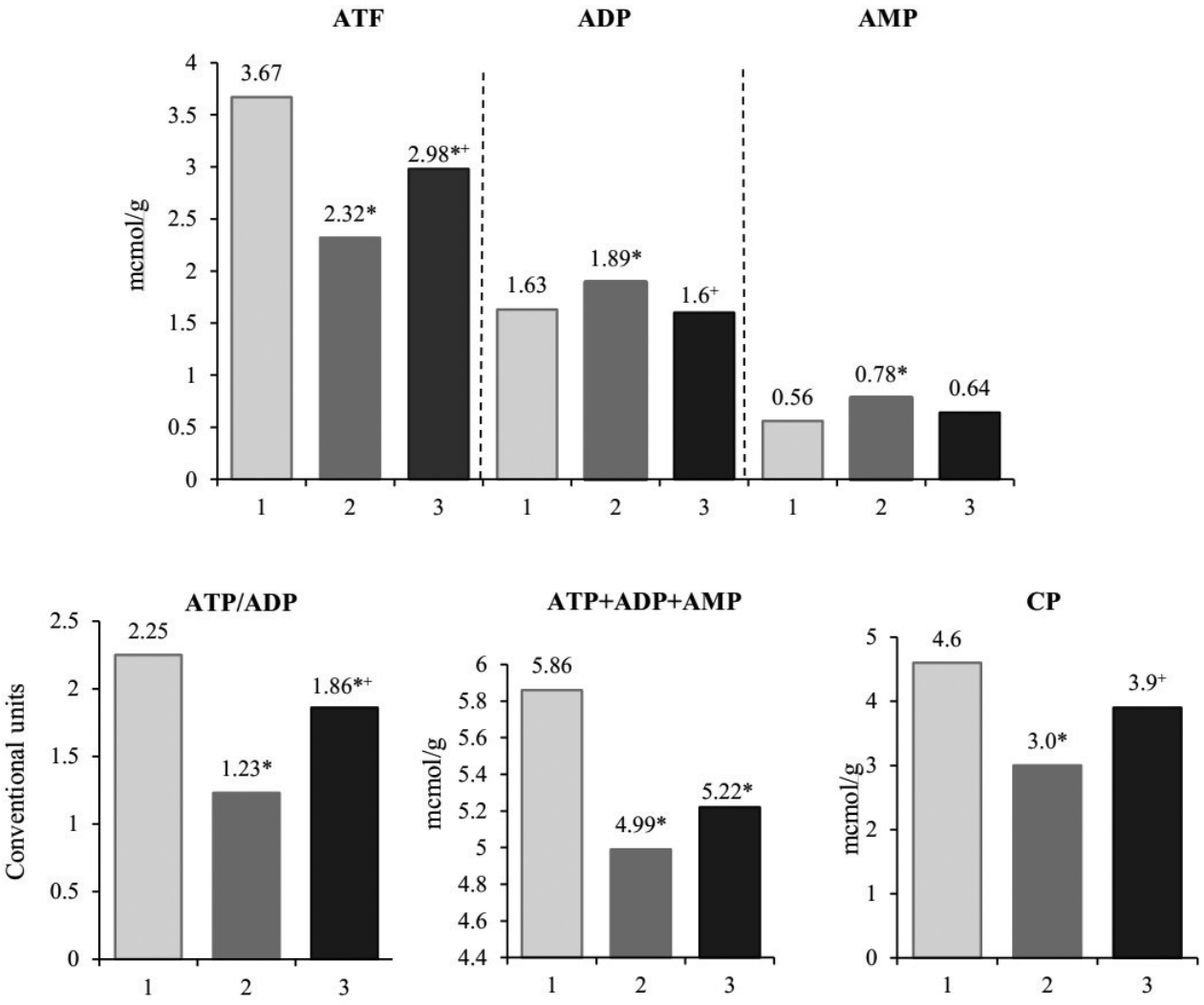


Figure 5. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the level of ATP, ADP, AMP, ATP/ADP, and the sum of ATP+ADP+AMP, CP in the rat skin ischemia. **Note**: bar charts: 1 – intact skin, 2 – normal saline, control, 3 – combination of mafusol with rexod; * – the differences are statistically significant compared to the norm, $^{+}$ – compared to the control.

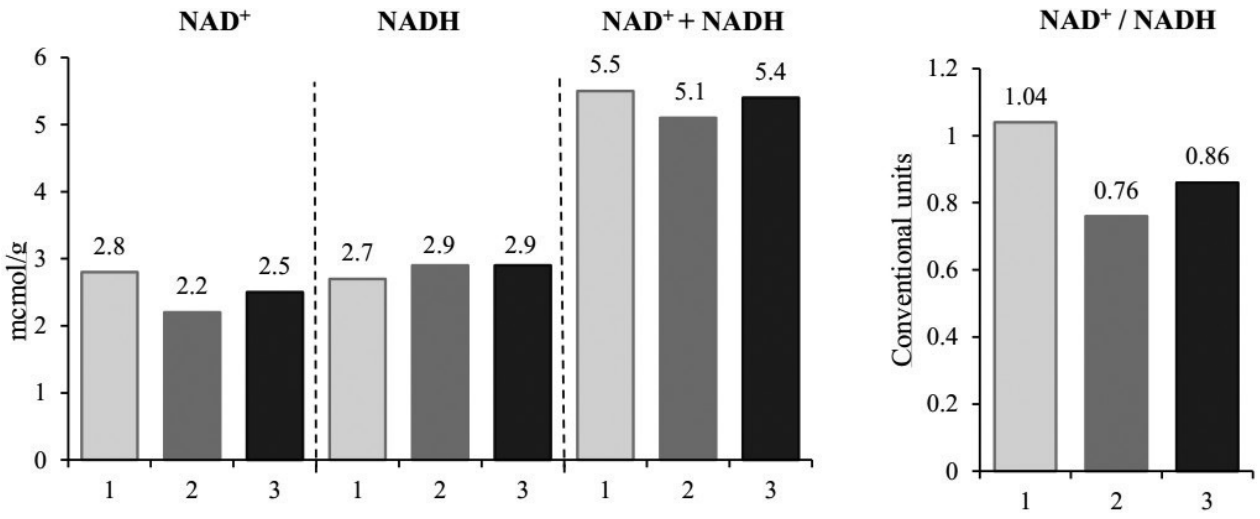


Figure 6. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the level of NAD^{+}, NADH, the sum of NAD^{+} + NADH, and the ratio of NAD^{+}/NADH in the rat skin ischemia.

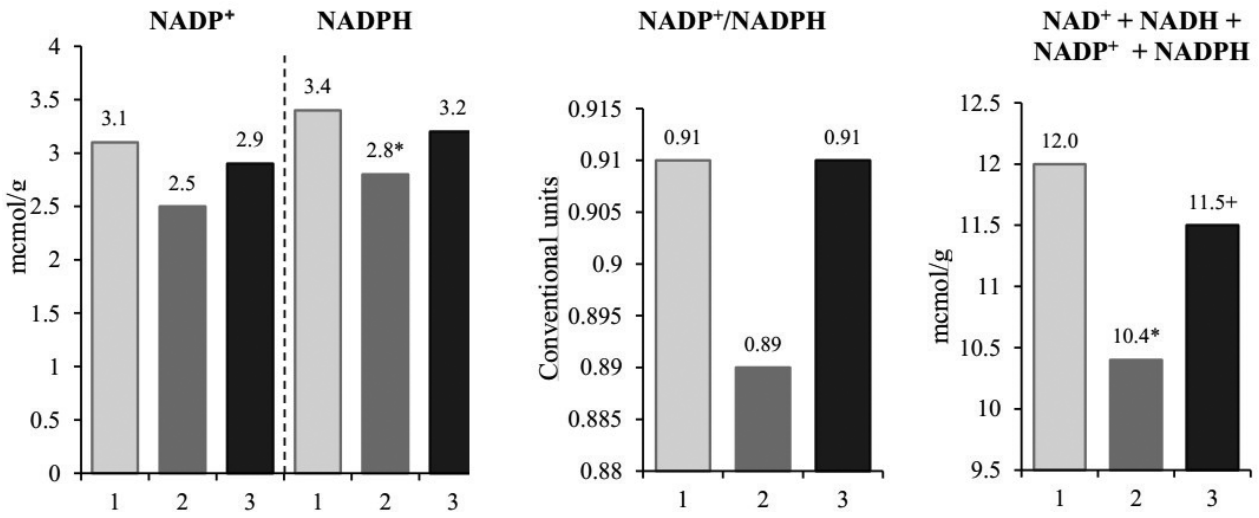


Figure 7. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the $NADP^+$, NADPH, $NADP^+$/NADPH ratio, and the sum of NAD^++NADH+$NADP^+$+NADPH in the rat skin ischemia. **Note**: bar charts: 1 – intact skin, 2 – normal saline, control, 3 – combination of mafusol with rexod; * – the differences are statistically significant compared to the norm, $^+$ – compared to the control.

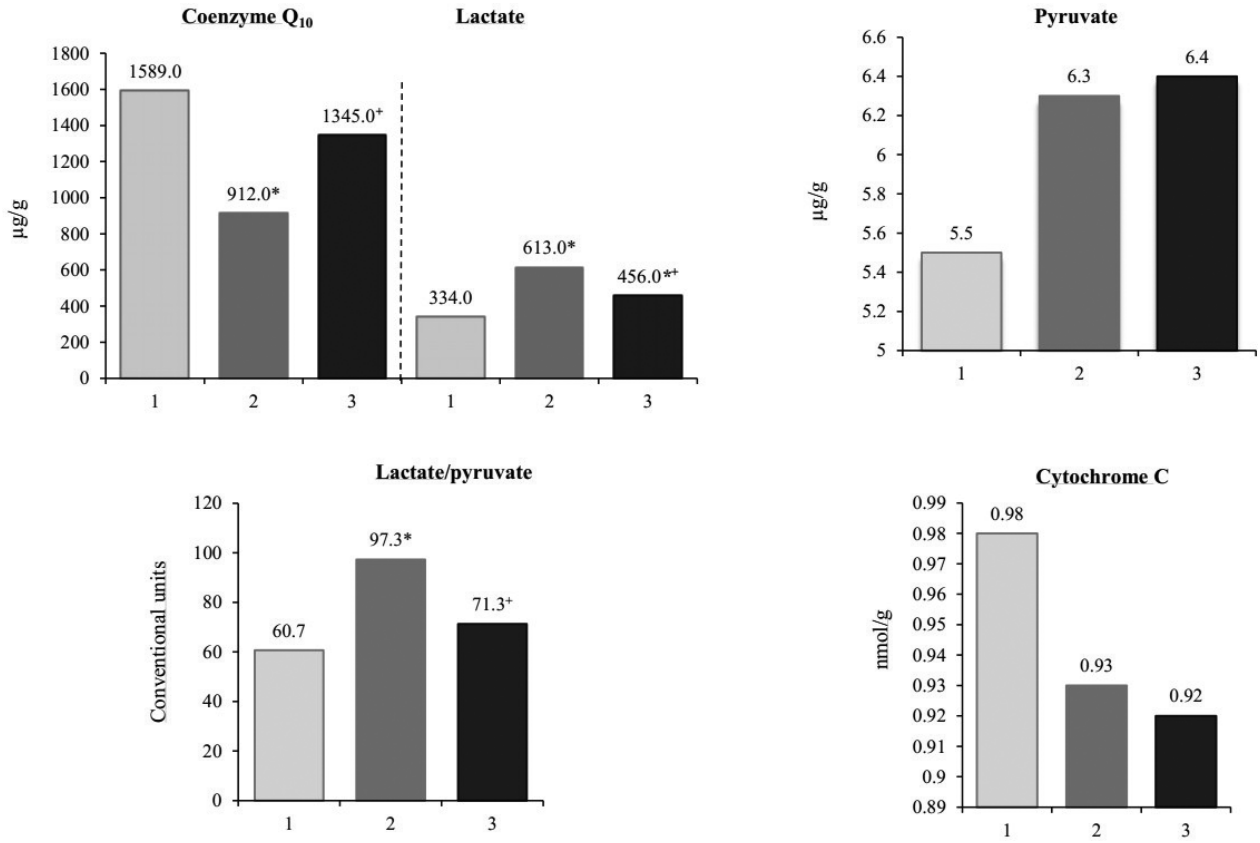


Figure 8. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the level of coenzyme Q10, lactate, pyruvate, cytochrome C, and the lactate/pyruvate ratio in the rat skin ischemia. **Note**: bar charts: 1 – intact skin, 2 – normal saline, control, 3 – combination of mafusol with rexod; * – the differences are statistically significant compared to the norm, $^+$ – compared to the control.

together with an increase in GP, indicates a pronounced antiperoxide effect of rexod.

The combination of mafusol (6.25 mg/kg, intravenously) with rexod (0.01 mg/kg, intraperitoneally) has a favorable effect mainly on the endogenous factors of the ADN, since it significantly increases the activity of the studied ADN enzymes. In addition, this combination reduces the rate of O_2^- generation, which can be considered as one of the mechanisms for limiting ROS formation and reducing the load on the ADN (Fig. 10).

The study of the effect of the medications and their combination on the intensity of the necrotic process un-

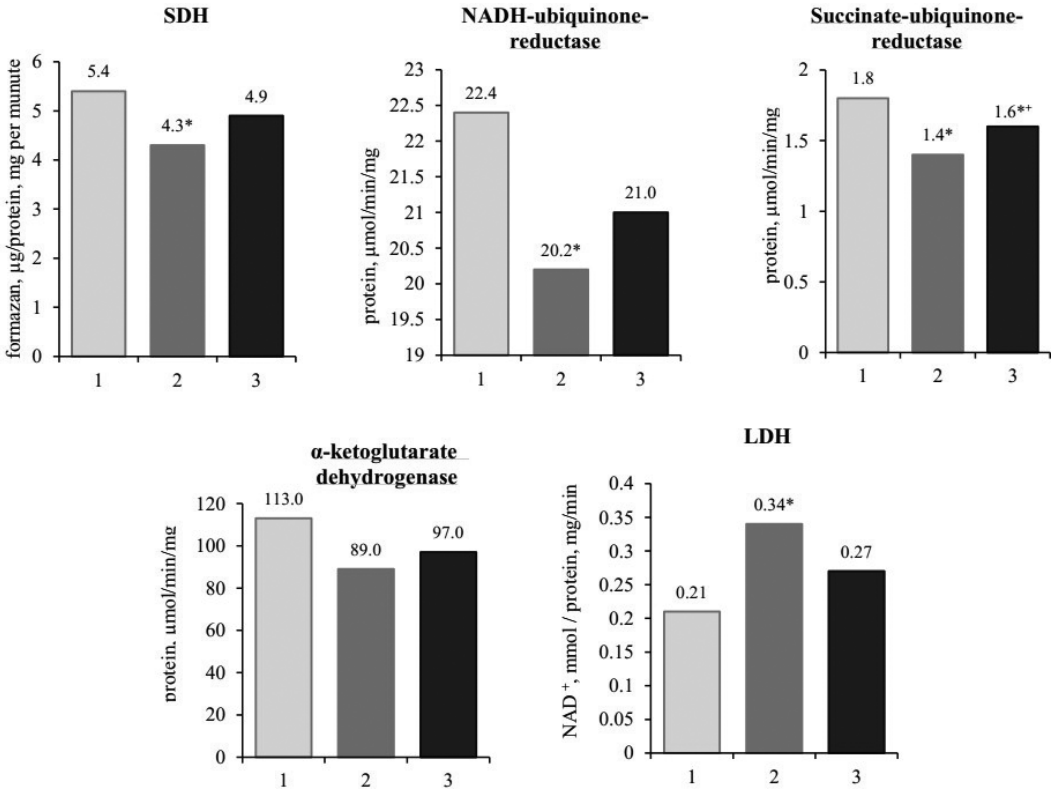


Figure 9. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the activity of SDH, NADH-ubiquinone reductase, succinate-ubiquinone reductase, α-ketoglutarate dehydrogenase and LDH in the rat skin ischemia. **Note**: bar charts: 1 – intact skin, 2 – normal saline, control, 3 – combination of mafusol with rexod; * – differences are statistically significant compared to the norm, $^{+}$ – compared to the control.

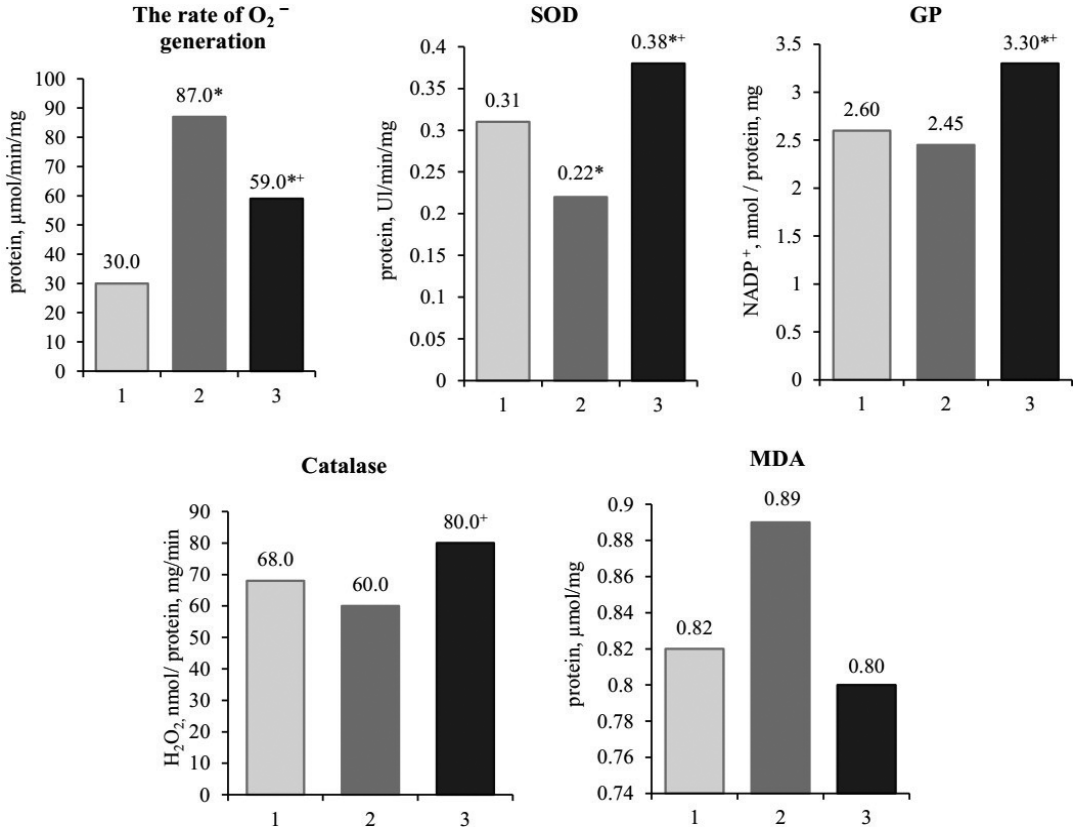


Figure 10. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the rate of O_2^- generation, SOD, GP, catalase activity, and MDA level in the rat skin ischemia. **Note**: bar charts: 1 – intact skin, 2 – normal saline, control, 3 – combination of mafusol with rexod; * – the differences are statistically significant compared to the norm, $^{+}$ – compared to the control.

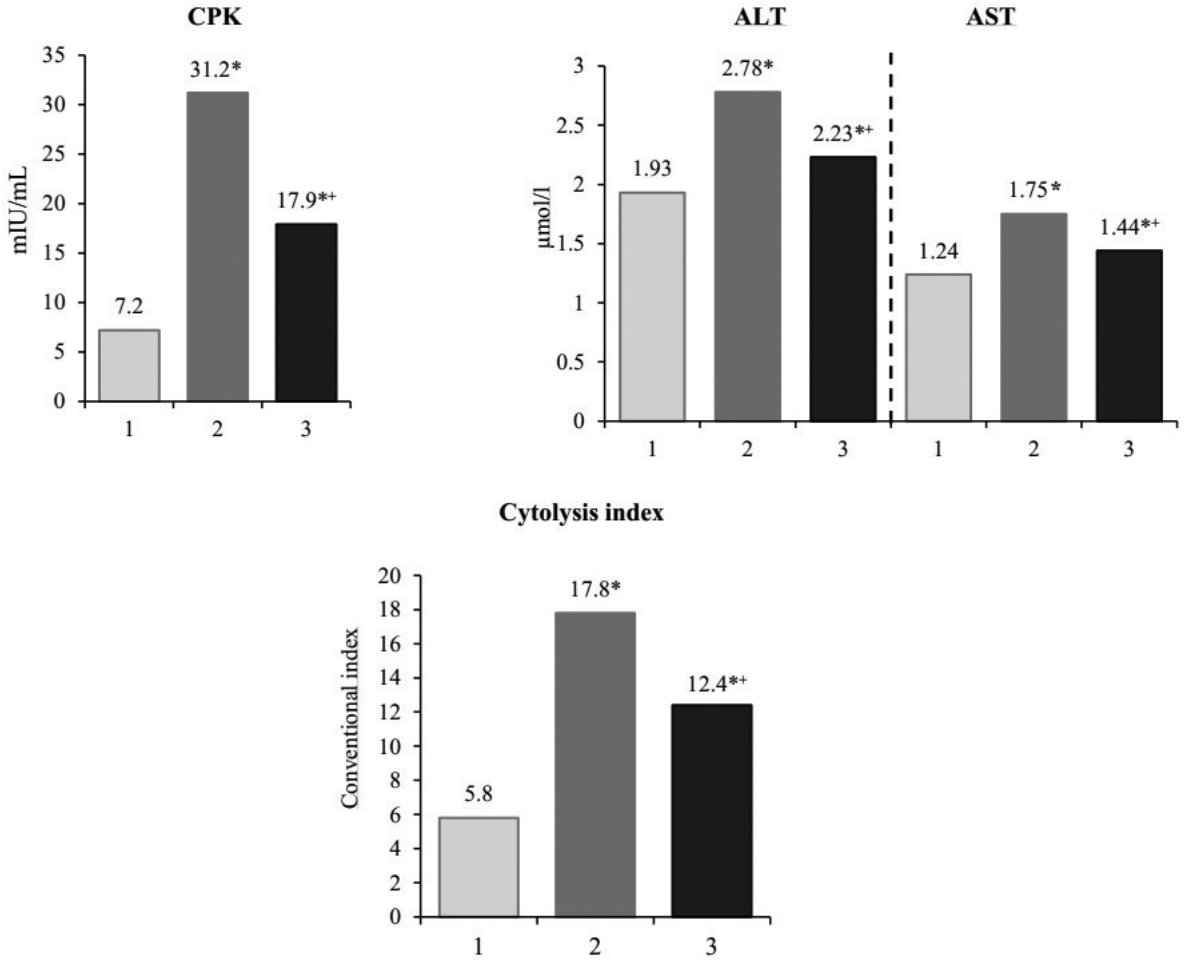


Figure 11. The effect of the combination of mafusol (6.25 mg/kg) with rexod (0.01 mg/kg) on the activity of CPK, ALT, AST, and the cytolysis index in the rat skin ischemia. **Note**: bar charts: 1 – intact skin, 2 – normal saline, control, 3 – combination of mafusol with rexod; * – differences are statistically significant compared to the norm, $^{+}$ – compared to the control.

der the accepted experimental conditions revealed that both mafusol (12.5 mg/kg, intravenously) and rexod (0.02 mg/kg, intraperitoneally) have a positive effect on the structure and function of the cell membranes and cells in general, showing a cytoprotective effect, which is characterized by a decrease in the activity of the enzymes CPK, ALT, and AST in the extracellular space. At the same time, the anti-cytolytic effect was more pronounced in mafusol, and evidenced in a decrease in the cytolysis index in addition to reducing the elevated level of the studied cellular enzymes.

Mafusol (6.25 mg/kg, intravenously) and rexod (0.01 mg/kg, intraperitoneally) in combination potentiate the metabolic effect of each other, which was reflected in a significant decrease in the activity of CPK, ALT and AST, as well as of the cytolysis index (Fig. 11).

Discussion

Rexod is able to reduce the acute toxicity of mafusol. The mechanism of the dermatoprotective action of mafusol, rexod, and, to a greater extent, of their combination may be associated with the ability of these medications to improve the MHC, to produce the antihypoxic effect and to activate energy processes in the skin, to normalize carbohydrate and lipid metabolism in AD, complicated by EHC, and to increase the reserve capacity of the ADN. In addition, the dermatoprotective effect of the mafusol+rexod combination is attributable to the ability of these medications to reduce the viscosity of the blood and to improve its rheological properties. Mafusol significantly increases the dermatoprotective activity of alprostadil when administered concurrently.

Conclusion

Combinations of mafusol with rexod and alprostadil can be recommended for clinical study as medications increasing the skin survival in reduced blood circulation resulted from traumatic injuries and diabetes complicated by atherosclerosis.

Conflict of interest

The authors declare no conflict of interest.

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