Research Article

The Dermatological Hydrogel Containing Fulvic Acids

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ABSTRACT

The composition of the dermatological hydrogel containing fulvic acids for the treatment of burn wounds is being proposed. It has been shown that wound healing was proceeded more intensively by contrast to control groups (I, II) on the 10th days, the burn area was decreased by 70%, the improving microcirculation in periwound area by using of thermal injury model on Wistar rats. The efficiency of the hydrogel was evaluated in the decreasing of LPO intensity and the MDA level, the increasing of SOD activity in plasma and in erythrocytes under the action of fulvic acids. Moreover, fulvic acids increased LDH activity in both direct and indirect reactions in comparison with control.

Keywords: Fulvic and humic acid, dermatological hydrogel, antioxidant and lactatedehydrogenase activity.

INTRODUCTION

Humic acids (fulvic, humic, hymatomelanic) isolated from soils, peat, natural waters, coal, mummies and other products of plants, bacteria and animals processing are of great interest for medicine and pharmacy.[1-3] Fulvicacids (FA) enriched in phenolic and carboxylic groups with a lower molecular weight and accordingly better solubility are the most perspective compounds.

The studies *in vitro*, *in vivo* and in clinical practice have proved their antimicrobial, anti-inflammatory, antitumor, antiulcer, reparative, wound and burns healing activities and other properties while the spectrum of diseases which are effectively treated by FA is continuously expanding.[4-6]

Biochemical conditions that can lead to many of the mentioned diseases are excessive production of the reactive oxygen species (ROS) and increased production of the free radicals (LPO). Potentiation of ROS production estimates disruption of energetic metabolism in cells, decreasing of SOD activity and changing in iron homeostasis. The presence of reactive groups such as catechol and phenolic hydroxyls in the composition of FA and HA determines their antioxidant properties similar to the other polyphenols, esters of gallic acids, tannins and ellagic acids effectively protecting the human erythrocytes against hemolysis under the action of ROS.[7-11] The protective effect of the above-mentioned compounds was also shown in the regulation of the lactate dehydrogenase (LDH) levelinerythrocytes.[12] The high level of the LDH is found in any stress situation and damages of skin, thermal injures wounds and others.

The object of this study is development of the dermatological hydrogel containing fulvic acids and investigation of its efficacy on the model of thermal back skin injury by rats.

MATERIALS AND METHODS

Fulvic acid derived from the peat of Nizhny Novgorod region (Beauty Land Company, Russia). The characteristics of fulvic acid: nitrogen content – 1.5 mmol-eq/g; content of acid groups c^s – 6.4 mmol-eq/g; hydrophobicity index HI – 0,89; water solubility – 30-70 mg/% (depending on related substances and mixture mode). Sodium hyaluronate (powder from vitreous body of bull, 99,8%, 1,63 MDa. CPN Spol.s.r.o, The Czech Republic; "Sigma", Product Number: H7630);tris-(oxymethyl)-amino methane (Trometamol; "Merck KGaA", Darmstandt, Germany); ethanol (95%; OOO "Hyppocrates", Samara); purified water (resistivity ≥18 Cm).

Composition of the dosage form «Fulvogel», %:

Sodium hyaluronate – 1.70; fulvic acid (water soluble form) – 0,06; methyl-4-hydroxybenzoate (nipagin) – 0,20; purified water – to 100,00. The water soluble form of fulvic acids was obtained by triturated fulvic acids with maltodextrine in presence of trisamine. The composition of this form: FA– 100 mg; trisamine – 100 mg; maltodextrine – 10 mg. The solubility of FA form – 50-70 mg/% whereas pH of the water solution should not exceed 8.0. 60 mg of the water soluble form of FA was used for gel.

Preparation: 0.2 g of nipag in was dissolved in 10 ml of purified water with stirring and heating up to 60°C. The nipagin solution was added to 70 ml of water and then 1,7 g of sodium hyaluronate was added. After swelling of the polymer during 12 hours the solution was stirred until

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a homogeneous colorless gel base. Fulvic acid (0.06 g of a soluble form) was added to the gel base and it was dissolved with stirring. Then the gel mass was made up to 100,0 by purified water.

Composition of the dosage form «Fulvogel+», %: sodium hyaluronate– 1,70; laevomycetin sodium succinate– 0,50; fulvic acid (water soluble form) – 0,06; methyl-4-hydroxybenzoate – 0,20; purified water – to 100,00.

Preparation: 0,2 g of nipagin was dissolved in 10 ml of purified water with stirring and heating up to 60°C. The nipagin solution was added to 70 ml of water and then 1,7 g of sodium hyaluronate was added. After swelling of the polymer during 12 hours the solution was stirred until a homogeneous colorless gel base. Fulvic acid (0,06 g of a soluble form) was added to the gel base and it was dissolved with stirring. 0,5g of laevomycetin sodium succinate was dissolved in 10 ml of water and it was added to the base of gel. Then the gel mass was made up to 100, 0 by purified water.

The wound healing activity of gels was studied in compliance with the guidance on experimental (preclinical) study of new pharmacological substances (A.N. Mironov, 2012). The experiment was made on white male Wistar rats ("Stolbovaya" nursery station of Federal State Institution of Science «Scientific Center of Biomedical Technologies of the Federal Medical and Biological Agency», Russian Federation, Moscow) with 250-300 g body weight. All animals were kept under standard conditions with free access to food and water. The rats were divided into 4 groups: I – intact (healthy rats, n=10), II – control (the rats with thermal injury, n=10), III and IV – experimental (n=5 in each groups). The boiling water burn of dehaired back surface (20%) was caused to the rats of II, III and IV groups under mixed anesthesia (Zoletil 60 mg/kg + Xyla 6 mg/kg), exposure – 3s. The rats of group III were treated topically by using "Fulvogel" (gel 1), the rats of group IV – by using "Fulvogel +"(gel 2). The animals were taken out of the experiment on the 10thday after injury by decapitation under mixed anesthesia.

Biomedical research

The blood stabilized by sodium citrate (1:9) was used for this study. The erythrocytes were washed two times by 0,9% NaCl solution then were centrifuged for 10 minutes at 1600 g. The level of malonic dialdehyde as a marker of lipid peroxidation in the plasma and erythrocytes was measured according to the M. Ukiyama, M. Mihara methods. The SOD activity (SOD, EC 1.15.1.1) in the hemolysate of washed red blood cells (1:10) was determined by inhibition of the auto oxidation product of adrenaline.[13] The lactatedehydrogenase activity (LDH, EC 1.1.1.27) was determined in the hemolysate of washed red blood cells (1:40). Catalytic activity of the LDH in direct reactions (LDH_{dir}) was estimated by using the 50 mM sodium lactate as a substrate; but the indirect reaction (LDH_{ind}) – by using the 23 mM sodium pyruvate.[14] Calculation of the specific activity of enzymes was made according to the modified Lowry method.[15]

Microcirculation state in intact skin and in thermal injury was fixed by laser Doppler flow metry (complex "LAKK-M"). Analyzing probe sets perpendicularly to the damaged area. Microcirculation (4 minutes) was estimated by microcirculation index (MI) which reflects average erythrocyte flow per unit tissue volume per unit time.

The Wavelet analysis (amplitude-frequency spectrum) was assessed by using program LDF allowed to calculate passive oscillations of respiratory (R) and cardiac (C) waves and active oscillations of myogenic (M) and neurogenic (N) ranges. The role of microcirculatory shunting was estimated by the measurement of shunt index (SI).

RESULTS AND DISCUSSION

Efficacy evaluation of "Fulvogel" and "Fulvogel+" was made in the experiment by using 4 groups. The control group I consisted of the intact animals ("healthy") and control group II included the animals that had been burned without treatment ("burn"). Experimental groups III and IV were represented by rats which were treated using "Fulvogel" and "Fulvogel +" accordingly. The disappearance of the eschars with a hard crust, the reducing the burn area by 80% was observed after treatment by "Fulvogel" (group III)on the 10th day, in contrast to the eschars that were retained in control group II ("burn")and the burn area was reduced only by 20%. Healing of the burn wounds by treatment of "Fulvogel +" occurred more intensively on the 10th day, decreased of the burn area - by 90%, the eschars disappeared (Figure 1a, b, c).

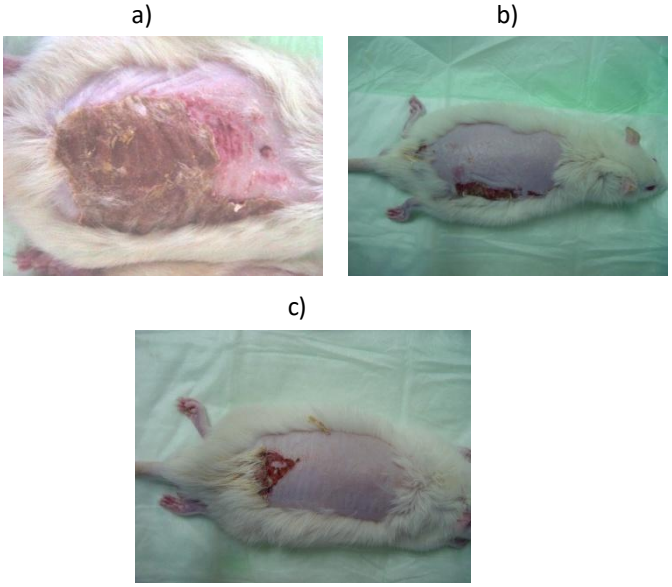

Figure 1: Healing of the burn wounds: a) control group II ("burn"); (b) under "Fulvogel" treatment during 10th day (group III); c) under "Fulvogel +" treatment during 10th day (group IV).

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The intensity of microcirculation, the activity of its regulatory components were studied taking into account the peculiarities of the intervals of frequency ranges of blood flow oscillations on the micro vessels of rats.[16,17]

It has been established in terms of the assessment of local microcirculation that the application of "Fulvogel" provides the maintenance of microcirculation index (MI) in the periwound area at elevated level on the 10th day of the post-burned period. It characterizes the stimulation of regenerative processes. In contrast, the significant arrest of microcirculation in relation to control group II ("burn") was observed in the treatment by "Fulvogel +" but this value was close to the norm (control group I, "healthy")(Figure 2a). The neurogenic and myogenic components of microcirculation reflect a local regulation. The intensity of regulatory mechanisms was represented by neurogenic (N), myogenic (M), respiratory (R) and cardiac (C) components. The values of the neurogenic and myogenic components increased in comparison with control group II ("burn") and approach to the values of control group I ("healthy") that characterizes the effective local regulation of microcirculation in the periwound area(Figure 2b).

The analysis of the amplitude-frequency spectrum showed that the amplitude values of active and passive regulatory mechanisms in group III treated by "Fulvogel" increased in comparison with control group II ("burn") and were close to the values of control group I ("healthy") that characterizes the effective local regulation of microcirculation in the periwound area. The contribution of arteriolo-venular shunts in the microcirculation system is mostly expressed in group III treated by "Fulvogel" whereas in group IV treated by "Fulvogel +" the shunt index (SI) is comparable to control group I ("healthy") (Figure 2c).

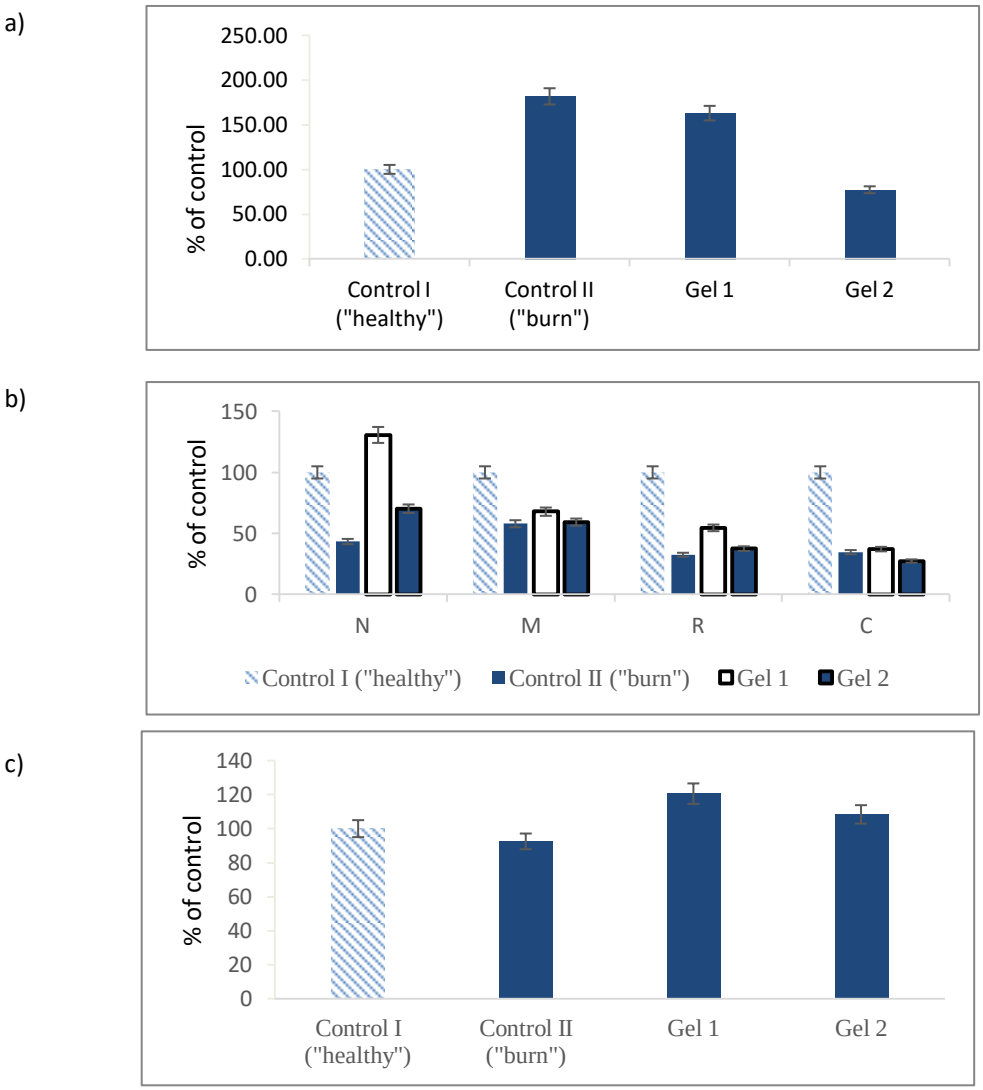

Figure 2: The values of microcirculation indexes of rats with thermal injury on the 10th day of the experiment: a) microcirculation index (MI) (% of control); b) intensity of regulatory mechanisms (% of control); c) shunt index (SI) (% of control).Legends: MI – microcirculation index; N – neurogenic component; M – myogenic component; R – respiratory component, C – cardiac component.

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In general, it may be noted the positive effect of the components of "Fulvogel", specifically fulvic acids, on the efficiency of microcirculation in the periwound area. It determines the effective stimulation of regeneration processes.

Biochemical indexes were determined in the blood plasma and in the erythrocytes of rats. The effect of fulvic acids in salt form with trisamine in "Fulvogel" on non-enzyme antioxidant activity in blood plasma appeared in the decrease in the LPO intensity determined by the chemiluminescence method in comparison with control group II ("burn"). The total antioxidant and ROS production indexes were changed insignificantly (Figure 3a, b). In addition, the level of malonicdialdehyde (MDA) as a secondary metabolite of LPO was decreased by 40% mostly pronounced by the treatment of "Fulvogel" (group III) (Figure 3c).

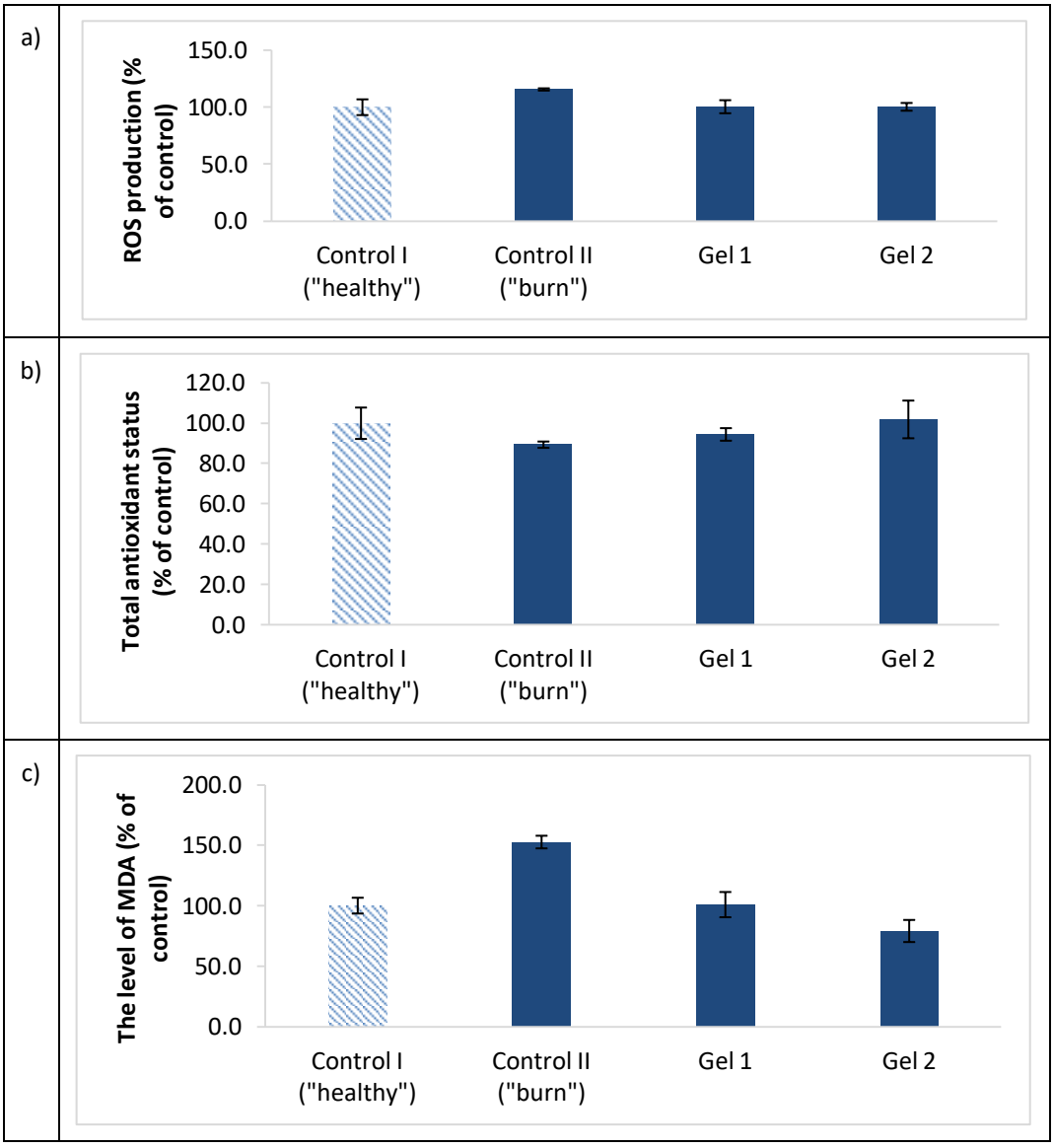

Figure 3. Antioxidant properties of hydrogel with fulvic acids on the model of thermal back injury inthe blood plasma of rats: a) ROS production (% of control); b) total antioxidant status (% of control);c) the level of MDA (% of control).

The pro-oxidant effect in erythrocytes was observed under the action of "Fulvogel" (group III) with respect to radical oxidation (Figure 4a). The intensity of LPO in erythrocytes increased by 20% compared to control group II ("burn"). In contrast, the addition of laevomycetin sodium succinate to gel 1 (group IV, gel 2), the intensity of LPO was significantly reduced compared to control group II ("burn") (Figure 3a).

The MDA level in erythrocytes was increased under the treatment of "Fulvogel" (group III) and "Fulvogel +" (group IV) in comparison with control group II ("burn") (Figure 4b). At the same time, the SOD activity significantly increased (Figure 4c).This fact characterizes fulvic acids as antioxidants with a suitable redox potential similar to polyphenols such as gallic acids. In addition, the antioxidant effect is probably due to the action of fulvic acids as effective chelators of iron and other transition metals.

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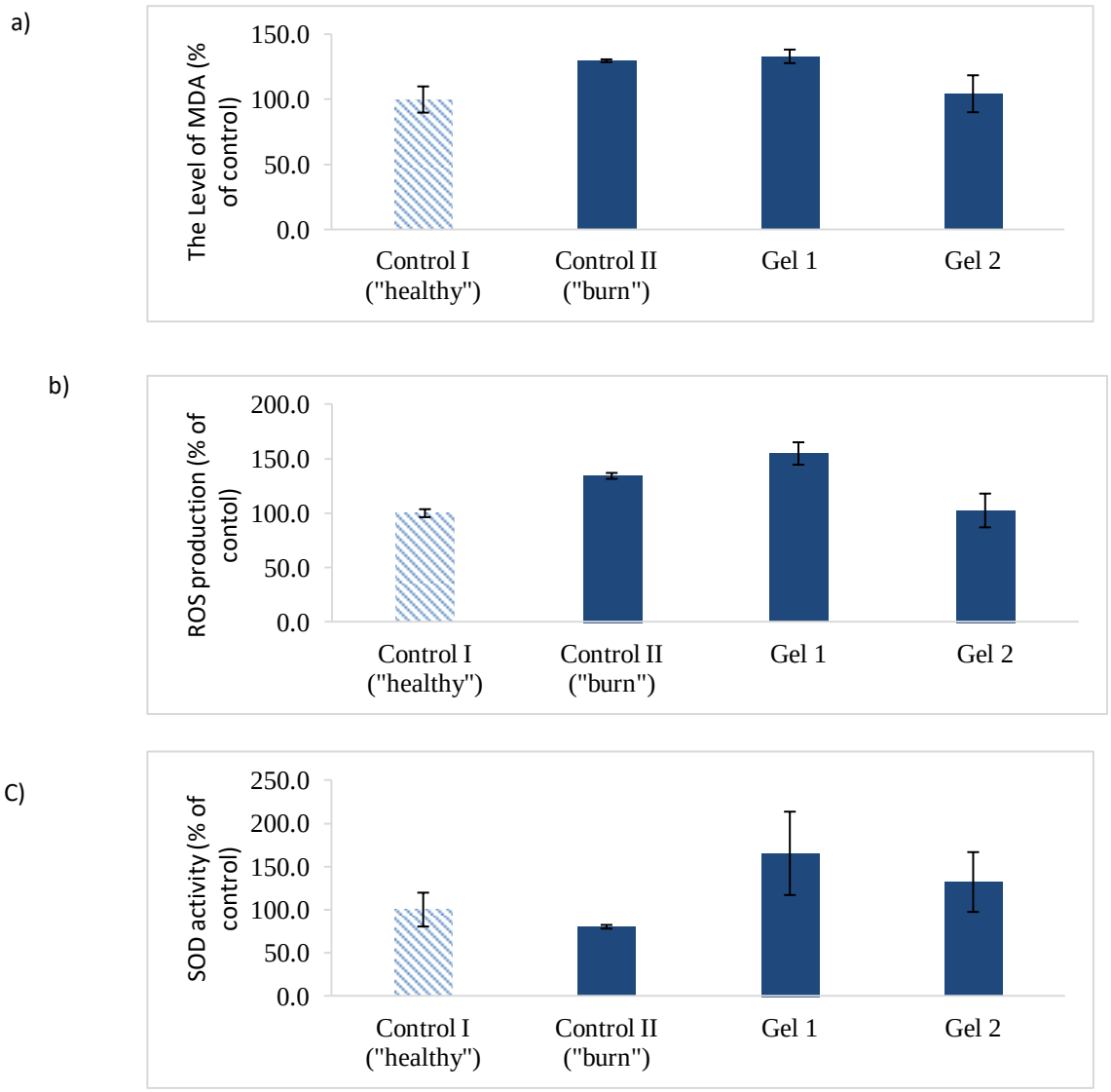
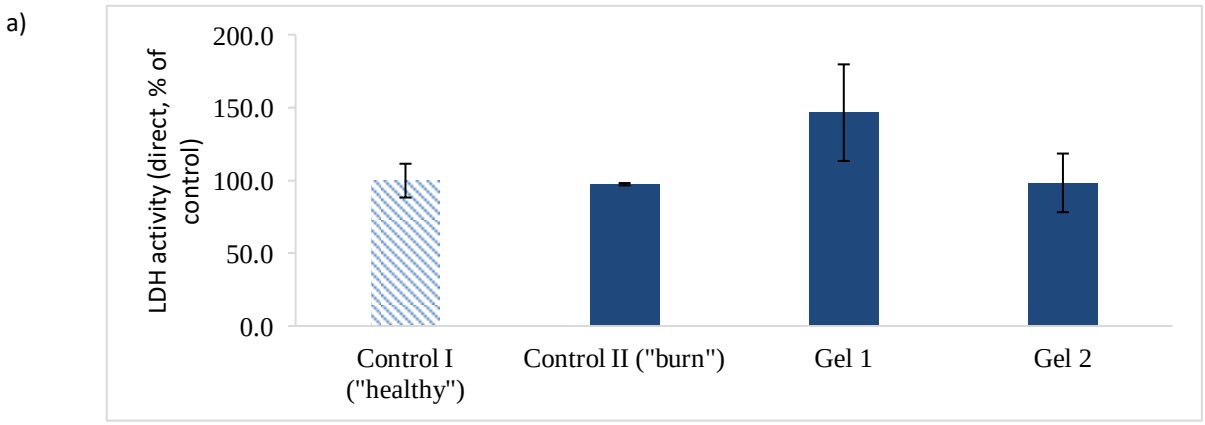


Figure 4. Antioxidant properties of hydrogel with fulvic acids on the model of thermal back injury inthe erythrocytes of rats: a) ROS production (% of control); b) the level of MDA (% of control); c) SOD activity (% of control).

The energy metabolism in cells was estimated by the lactatedehydrogenase activity (LDH) in bothdirect and indirect reactionsNAD/NADH^{+}. In the direct reaction pyruvate is formed from lactate which can be used in the Krebs cycle under aerobic conditions with the formation of the main energy source - adenosine triphosphate. The indirect reaction of the LDH led to the formation of lactate from pyruvate and characterizes the degree of the expression of anaerobic process in the cell (Figure 5 a,b).



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b)

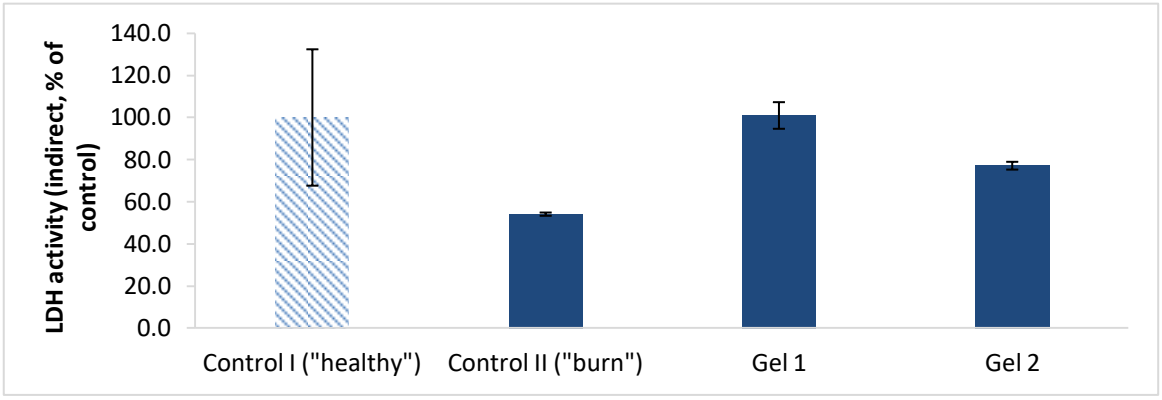

Figure 5. The lactatdehydrogenase activity in (a) direct and in (b) indirect reactions

From the data of Figure 5, it can be concluded that fulvic acids contribute to the increasing of the LDH activity in both direct and indirect reactions compared to control group II ("burn") that is why they act as a regulator of energy metabolism in the cell.

CONCLUSION

The composition of the wound and burns healing hydrogel "Fulvogel" containing fulvic acids as an active ingredient is proposed.

In the experiments *in vivo* on rats the "Fulvogel" effectiveness on the model of thermal back skin injury was proved: the burn area was reduced by 80%, the hard eschar disappeared, and effective epithelization was noted on the 10^{th}day. Moreover, the improvement of biochemical indexes in blood plasma and in erythrocytes was shown. The LPO reduction in plasma is confirmed by the decrease of the malonicdialdehyde level (by 40% compared to control group II, "burn") and the increase of superoxide dismutase activity in erythrocytes (by 85% compared to the control - burn). The biological benefits of energy metabolism in erythrocytes were manifested by the increase of lactate dehydrogenase activity in both direct and indirect reactions (≈ 50%).

"Fulvogel" can be offered for cosmetic purposes as a product to prevent aging and withering of the skin. The formula "Fulvogel +" including leavomycetin sodium succinate allows it to be used in the treatment of burn wounds.

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