

EXPERIMENTAL INVESTIGATION OF THE BURN-HEALING PROPERTIES OF A NOVEL CREAM BASED ON POLYPRENOLS DERIVED FROM *GINKGO BILOBA L.*

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In recent years, the number of emergencies, armed conflicts, and household injuries has increased, which has elevated the risk of thermal burns for a broad segment of the population. Design and testing of new, safe and efficient burn-healing drugs remains a relevant task. This study aimed to evaluate the burn — healing effects of a new cream containing polyphenols isolated from *Ginkgo biloba L.* in a rat thermal-burn model. The cream's active ingredient is a highly purified ($\geq 95\%$) *Ginkgo biloba L. polyphenols*, and the excipients are lipids (phosphatidylcholine, phosphatidylserine) with tocopherol antioxidants. The experimental animals were 32 male Wistar rats weighing 280–320 g. We evaluated the effect of the new polyphenol cream and the reference preparations methyluracil and dexpanthenol on the dynamics of the skin lesion area in rats with grade III-B skin burns. The experiment showed that, on day 21 after the burn, the new cream reduced the wound area in rats by 2.3 times compared with the controls. Morphological examination showed that the new cream had a 1.3-fold better burn-healing effect than the comparison drug methyluracil (dioxomethyltetrahydropyrimidine). Further preclinical study of the cream as a potential burn and wound healing agent seems promising.

Keywords: polyphenols, *Ginkgo biloba L.*, cream, thermal burn, burn-healing effect, rats

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ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ ПРОТИВООЖГОВОГО ДЕЙСТВИЯ НОВОГО КРЕМА НА ОСНОВЕ ВЫДЕЛЕННЫХ ИЗ *GINKGO BILOBA L.* ПОЛИПРЕНОЛОВ

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Возросшее за последние годы количество чрезвычайных ситуаций, вооруженных конфликтов, а также бытовой травматизм обуславливают повышенный риск получения термических ожогов у достаточно широкой группы людей. Разработка и изучение новых эффективных и безопасных препаратов с противоожоговым действием в настоящее время продолжает оставаться актуальным. Целью исследования было изучить противоожоговое действие нового крема на основе полипенолов, выделенных из *Ginkgo biloba L.*, на модели термического ожога у крыс. На основе полипенолов был разработан новый крем следующего состава: действующее вещество — высокоочищенная субстанция полипенолов *Ginkgo biloba L.* с их содержанием не менее 95%, вспомогательные вещества из группы липидов (фосфатидилхолин, фосфатидилсерин) и антиоксиданты из группы токоферолов. Эксперименты выполнены на 32 крысах-самцах линии Вистар массой 280–320 г. Оценено влияние нового крема с полипенолами и препаратов сравнения метилурацила и декспантенола на динамику площади поражения кожи у крыс с термическим ожогом III Б степени. Показано, что на 21-е сутки после ожога новый крем уменьшал площадь поражения кожи крыс по сравнению с контролем в 2,3 раза. По результатам морфологической оценки по выраженности противоожогового действия данный крем превосходит препарат сравнения метилурацил (диоксометилтетрагидропиримидин) в 1,3 раза. Отмечена перспективность дальнейшего доклинического изучения в качестве потенциального противоожогового и ранозаживляющего средства.

Ключевые слова: полипенолы, *Ginkgo biloba L.*, крем, термический ожог, противоожоговое действие, крысы

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A burn is a severe tissue injury most often caused by exposure to heat; it can impair basic bodily functions and may lead to disability or death [1–3]. In recent years, the number of emergencies, armed conflicts, and household injuries has increased, which elevated the risk of thermal burns for a broad segment of the population. Despite the intensive development of regenerative medicine, successful treatment of burn wounds is still a difficult task. The healing process is slowed by serious metabolic disorders, diseases of the cardiovascular system, wound infection, etc., as well as insufficient effectiveness of the drugs used [2, 4]. Thus, design and testing of new, safe and efficient burn-healing drugs remains a relevant task.

We selected polyphenols, long-chain isoprenoid alcohols present in bacteria, plants, and mammals, as the basis for a new burn-healing drug. The varied pharmacological properties of these compounds are determined by their unique chemical structure and homologous polymers. Capable of regulating biological processes, polyphenols participate in the synthesis of glycoproteins of cell membranes, have pronounced hepatoprotective, neuroprotective, and cardioprotective effects, improve cognitive function, possess antioxidant, lipid-lowering, anti-inflammatory, immunomodulatory, antibacterial, antiviral, and antitumor activities, stimulate protein synthesis, and promote regeneration [5–9].

Polyphenols are mainly found in coniferous trees such as various species of spruce (*Picea spp.*), fir (*Abies spp.*), and pine (*Pinus spp.*), as well as in Ginkgo biloba (*Ginkgo biloba L.*). As for the quality of the derived substance, *Picea abies L.* has shorter polyphenols compared to that obtained from *Ginkgo biloba L.* [10].

We designed a new cream that has highly purified ($\geq 95\%$) *Ginkgo biloba L.* polyphenols as the active ingredient, lipids (phosphatidylcholine, phosphatidylserine) as excipients, and tocopherols as antioxidants.

Thus, this study aimed to evaluate the burn-healing effects of a new cream containing polyphenols isolated from *Ginkgo biloba L.* in a rat thermal-burn model.

METHODS

For the experiments, we took 32 male Wistar rats weighing 280–320 grams (Andreevka branch of the Scientific Center for Biomedical Technologies of the Federal Medical-Biological Agency, Moscow Region, Russia). The animals were maintained in a controlled vivarium (temperature 20–22 °C, relative humidity 40–50%, 12-hour light–dark cycle) and had access to standard granular feed and filtered tap water ad libitum. Before the experiment, all rats were quarantined for 14 days.

Under general anesthesia (zoletil and xylazine, administered IM), we inflicted grade III-B thermal skin burns at the withers of the animals. The procedure was performed in accordance with the Guidelines for the Experimental (Preclinical) Study of New Pharmacological Substances (2012) [11]. The burns were produced by a 100 g stainless-steel weight (contact diameter 2.3 cm) heated to 120 °C and placed on depilated skin for 15 s. The animals received no anesthetics after the induction of the burn and during the follow-up period.

The rats were divided into four groups of eight animals each: C — control (burn without treatment), M — methyluracil, D — dexpanthenol, and PP — the new polyphenol-based cream. The treatment phase lasted 21 days; the study cream and the comparator medications — dioxomethyltetrahydropyrimidine (10% methyluracil; Nizhpharm, Russia) and dexpanthenol (5% panthenol 3D; Zelenaya Dubrava, Russia) — were applied daily at a dose of 0.15 g. We selected dioxomethyltetrahydropyrimidine and dexpanthenol as comparator drugs because they act

on the wound differently: dioxomethyltetrahydropyrimidine has anabolic properties and normalizes nucleic acid metabolism, whereas dexpanthenol supplies pantothenic acid and stimulates coenzyme A synthesis. Both agents are also widely used in clinical practice. Burn wound healing was monitored daily. The wound area was measured every 7 days and analyzed with ImageJ (NIH; Laboratory of Optical and Computational Instruments, University of Wisconsin, USA).

On day 21, the animals were euthanized in a CO₂ chamber. Skin from the burn area was excised, fixed in 10% neutral buffered formalin, and then processed and embedded in paraffin according to standard protocols. Morphological examination was performed using light microscopy. The 5- μ m-thick sections were cut on an Accu-Cut SRM rotary microtome (Sakura, Germany) and stained with hematoxylin and eosin. Slides were examined with a universal research microscope (Olympus, Japan) at low (40 $\times$) and high (400 $\times$) magnification, and images were captured with an Olympus UC30 (Olympus, Japan) camera.

To objectively assess morphological changes on day 21 after the burn, we examined the slides and scored them using a quantitative, points-based system across the following criteria:

- presence of mononuclear leukocytes, newly formed vessels, and adipocytes in the dermis: 0 — absent; 0.5 — single cells; 1 — moderate; 2 — marked/strong;
- formation of crusts or loose connective tissue or fibrous connective tissue: 0 — absent; 0.5 — present;
- formation of skin layers at the injury site: 0 — no layering; 0.5 — layering present, but dermis and epidermis not formed; 1 — dermis and epidermis formed;
- formation of the epithelium at the injury site: 0 — absent; 0.5 — partially present; 1 — fully formed.

Statistical processing of the results was performed in BioStat Pro 7. The normality of the data distribution was checked with the Shapiro–Wilk test. Since the distribution in the samples was normal or close to normal, the significance of the differences between the experimental groups was determined using the Student's t-test. The results were given as a mean and an error of mean ($M \pm m$). The differences were considered significant at $p < 0.05$.

RESULTS

Within 24 hours of sustaining a grade III-B skin burn, the animals formed a small, dry, dark red scab with smooth edges at the wound site. In the control group, the area of the injury was 381.6 ± 19.9 mm², and in the M, D, and PP groups it was 464.4 ± 34.6 , 441.8 ± 22.3 and 426.2 ± 11.1 mm², respectively. The color and shape of the scab remained unchanged throughout the experiment.

Seven days after the burns were inflicted, the damaged skin area in all groups did not differ significantly from day 1 measurements, which served as baseline (100%) (Fig. 1).

After 14 days, the injury area in all groups decreased significantly compared to baseline; the differences between groups M, D, PP, and control were not significant (Fig. 1).

The wound healing effect of the studied cream was the greatest on day 21 of the experiment: the area of the injury in the PP group was 2.3-fold smaller than in the control group ($p < 0.05$) (Fig. 1). Some animals had the scab peeling off completely, revealing healed wound.

Methyluracil and dexpanthenol were less effective: the results of application of the former did not significantly differ from the controls, and the latter reduced the damaged skin area by 1.7 times ($p < 0.05$) compared with the control group.

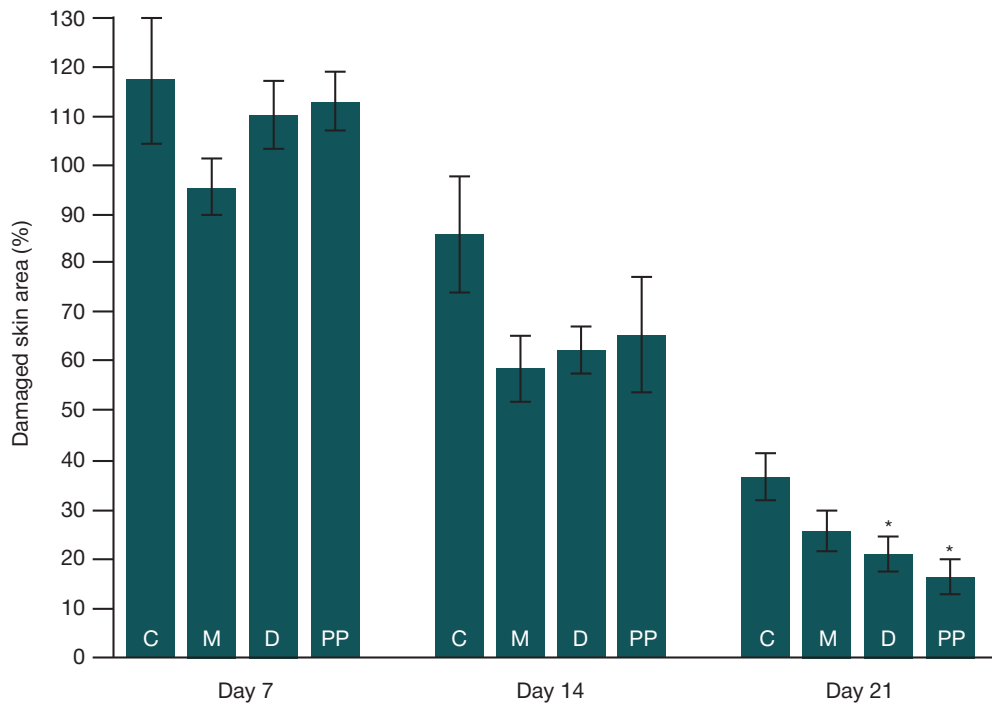


Fig. 1. The effect of the new polyphenol cream versus methyluracil and dexpanthenol ointments on skin lesion area dynamics in rats with grade III-B thermal burns ($M \pm m$). The damaged skin area on day 1 after the burn was taken as baseline (100%). Here and in Figures 2 and 3: C — control; M — methyluracil; D — dexpanthenol; PP — the new polyphenol-based cream. Significance of differences with the control: * — $p < 0.05$ (Student's *t*-test)

All animals in the respective groups retained the scab on their wounds.

Morphological examination confirmed damaged skin area changes. On day 21, control group animals had the wound surface covered with a scab that was abundantly infiltrated by polymorphonuclear leukocytes. Regarding epithelialization, the wound edges had all layers of epithelial tissue, while in the center the epidermis had only one or two layers and covered an insignificant portion (up to 20%) of the injury surface. We also noted beginning of formation of dermis. Beneath the scab, connective tissue infiltrated by leukocytes (mainly mononuclear cells and macrophages) contained a moderate to large number of newly formed vessels. Similar changes were observed in methyluracil and dexpanthenol groups, but the animals there had more newly formed vessels in the connective tissue beneath the scab (Fig. 2).

In the PP group, some animals had wounds completely covered by epithelium, beneath which there was connective tissue containing a few newly formed vessels and showing mild infiltration with leukocytes (mononuclear cells and macrophages). Other rats exhibited a scab abundantly infiltrated by polymorphonuclear leukocytes. Epithelialization covered 30% of the wound surface, with full-thickness layers present at the wound edges and single- to double-layered epidermis in the center. Connective tissue infiltrated by leukocytes with a large number of newly formed vessels was detected under the scab.

On day 21 after the burn, the assessment of the degree of morphological changes in the control group yielded 4.1 ± 0.5 points. The results registered in the M and D groups were not significantly different from those seen in the controls (Fig. 3). The new polyphenol cream produced significantly better wound-healing scores than the control and methyluracil groups (1.4- and 1.3-fold increases, respectively; $p < 0.05$) (Fig. 3).

DISCUSSION

Our results are consistent with the literature data. For example, it was previously demonstrated that polyphenols isolated from

Alcea nudiflora exhibit burn-healing and wound-healing activity in rats with grade III-A and III-B thermal burns and with full-thickness incisional and linear wounds. The application of these polyphenols promoted earlier coverage of the wound and made the scar tissue significantly less dense; the substance also enhanced epithelialization, formation of granulation tissue, and collagenesis. The polyphenols tested were more effective than sea-buckthorn oil: scabs detached faster than in controls, and proliferative and biosynthetic processes in epidermal and dermal cells were significantly stimulated. Studies involving rats with alloxan diabetes mellitus have shown that topical application of polyphenols promotes restoration of the key indicators of protein, carbohydrate, and lipid metabolism in epidermal cells, and helps form a more durable scar [12, 13]. In a rat model, individual polyphenols isolated from the leaves of *Vitis vinifera* subsp. *silvestris* (wild grape) and a whole polyphenolic fraction containing tocopherols and phytosterols produced a pronounced healing effect on clean, full-thickness planar skin wounds that exceeded the effect of sea-buckthorn oil [14].

In a study involving patients with burns, purulent wounds and other purulent-inflammatory diseases of the skin and soft tissues (various localizations), abisil, a 20% oil solution for topical and external use containing Siberian fir (*Abies sibirica* L.) terpenoids and possessing experimentally confirmed wound-healing properties, had a positive clinical effect in 86.5–96.3% of patients [15].

Regarding wound-healing mechanisms, several researchers believe that polyphenols activate plastic processes in the cellular structures of most tissues, suppress oxidative stress, increase the activity of enzymes involved in the cell's natural antioxidant defense, replenish dolichol phosphate, and promote its participation in the dolichol phosphate cycle, an important metabolic link in cell regeneration, differentiation, and proliferation [16, 17]. In addition, polyphenols increase the fluidity and permeability of biological membranes by changing the structure of fatty acids, thus stimulating metabolic processes and increasing cell viability [18].

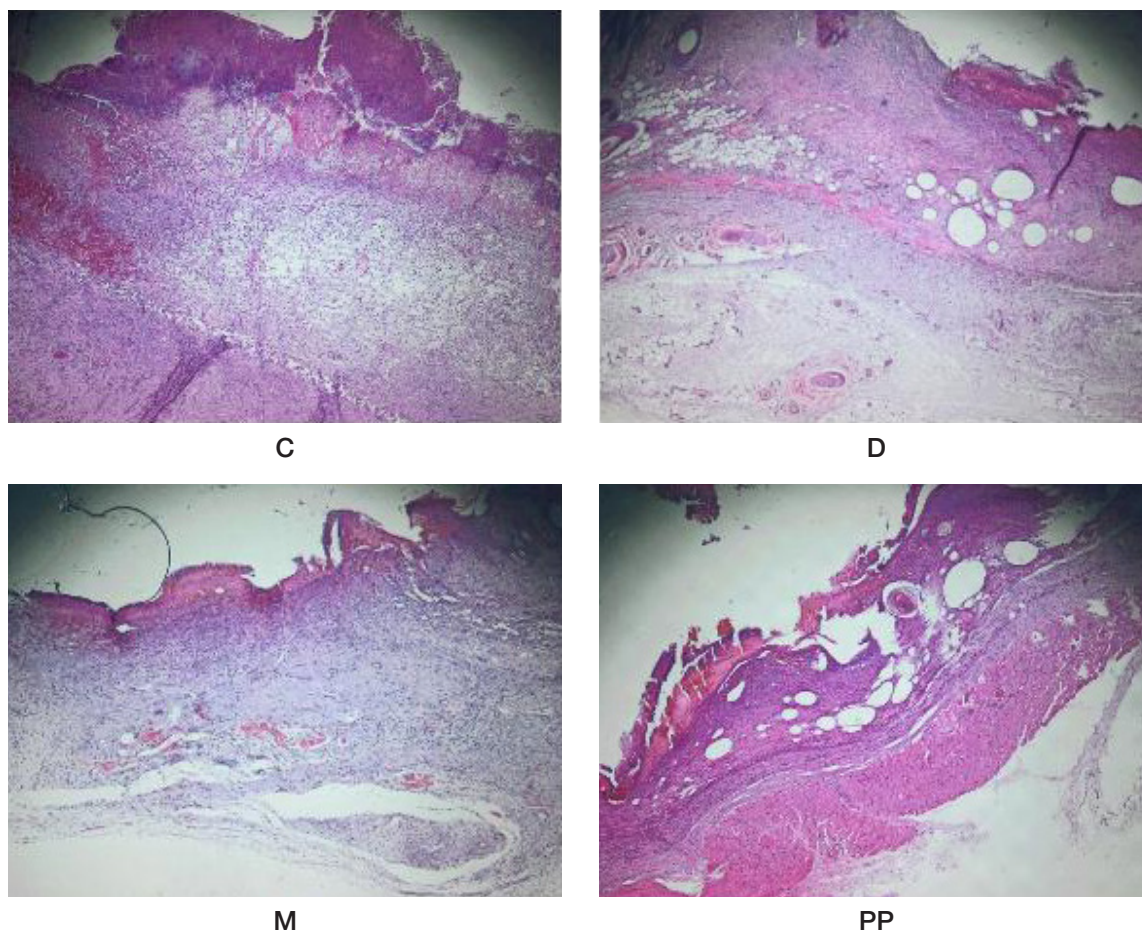


Fig. 2. Morphological changes in the skin of rats, day 21 after burn (hematoxylin and eosin staining; 10×4)

Regarding the efficacy of comparator drugs, the results of this study are consistent with the findings of other researchers. In a planar rat skin wound model, methyluracil was found to completely heal the injury only by days 24 to 29 of treatment, and the effect was not observed in all animals [19]. Moreover, the skin slides exhibited numerous distended vessels, suggesting incomplete repair, and a thinned epithelium; in some areas, the epithelium and dermis had not been restored at all. In the dermis, the researchers observed lymphoid infiltration, hemosiderosis, and an absence of leukocytes and necrotic

debris. As in our study, dexpanthenol did not promote scab detachment in a rat model of grade III-A burns, and complete wound healing occurred only by day 25 [20]. The findings of another study with similar experimental conditions did not differ significantly: on day 21 post-burn, dexpanthenol did not facilitate scab detachment or complete wound healing [21].

It should be noted that the experimental thermal burn model used in this study is officially recommended for investigating drug effects [11]. However, direct extrapolation of these results to humans requires careful analysis and consideration of

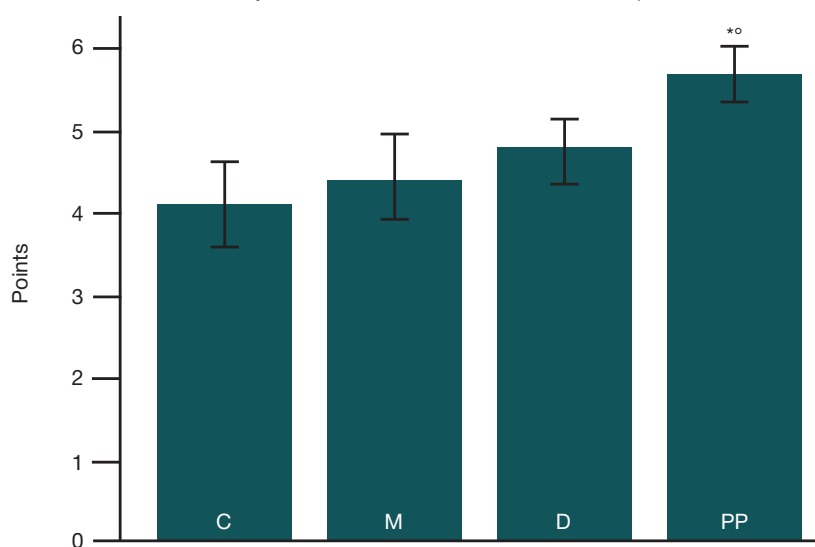


Fig. 3. The effect of the new polyphenol cream versus methyluracil and dexpanthenol ointments on the degree of histological changes in the skin (points, $M \pm m$) of rats with grade III-B thermal burns. The differences are significant compared to the control and methyluracil, respectively: * and ° — $p < 0.05$ (Student's *t*-test)

multiple factors, including methodological, pathophysiological, and biochemical aspects.

Thus, in rats with grade III-B thermal burns, a cream based on polyphenols isolated from *Ginkgo biloba* L. demonstrates therapeutic efficacy, reducing skin lesion area 2.3-fold by day 21 compared with the control and exceeding the effect of methyluracil by 1.3-fold, as confirmed by morphological evaluation. This cream should be considered for further preclinical study as a potential agent for burn treatment and wound healing.

CONCLUSIONS

In rats with grade III-B thermal burns, the new cream based on polyphenols isolated from *Ginkgo biloba* L. demonstrates burn-healing efficacy, reducing skin lesion area 2.3-fold by day 21 compared with the control. In terms of intensity of the healing effect, the cream exceeded that of methyluracil by 1.3-fold, as confirmed by morphological evaluation.

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